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A Flexible Approach to 1, 4-Disubstituted 2-Aminoimidazoles that Inhibit and Disperse Biofilms and Potentiate the Effects of β-Lactams against Multi-Drug Resistant Bacteria

Robert E. Furlani, Andrew A. Yeagley, Christian Melander

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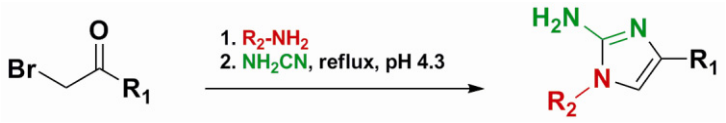
Graphical Abstract:



Compounds are described that inhibit and disperse MRSA biofilms, with the most potent compound displaying an IC_{50} of 4.14 μM. 2-Aminoimidazoles also suppress the MIC of oxacillin upwards of 4-fold against MRSA.

Highlights:

- **Development of a synthetic route to 1,4-substituted 2-aminoimidazoles**
- **Lead compounds inhibit both MRSA and multidrug resistant *Acinetobacter baumanii* biofilm formation**
- **Lead compounds disperse MRSA biofilm formation**
- **Lead compound suppresses the oxacillin MIC against MRSA 4-fold**

A Flexible Approach to 1, 4-Disubstituted 2-Aminoimidazoles that Inhibit and Disperse Biofilms and Potentiate the Effects of β-Lactams against Multi-Drug Resistant Bacteria

Robert E. Furlani, Andrew A. Yeagley, and Christian Melander*

Abstract:

The pyrrole-imidazole alkaloids are a 2-aminoimidazoles containing family of natural products that possess anti-biofilm activity. A library of 1,4-disubstituted 2-aminoimidazole/triazoles (2-AITs) was synthesized, and its anti-biofilm activity as well as oxacillin resensitization efficacy towards methicillin resistant Staphylococcus aureus (MRSA) was investigated. These 2-AITs were found to inhibit biofilm formation by MRSA with low micromolar IC_{50} values. Additionally, the most active compound acted synergistically with oxacillin against MRSA lowering the minimum inhibitory concentration (MIC) four-fold.

1. Introduction:

A biofilm can be defined as a matrix enclosed population of microorganisms that adhere to a biological or non-biological surface.[1] Bacteria within a biofilm are upwards of 1000-fold more resistant towards antibiotics than their planktonic counterparts.[2] The National Institutes of Health have estimated that over 80% of microbial infections in the human body are a result of biofilms.[3] As a result, bacterial biofilms can represent a significant impediment to the treatment of bacterial infections as they are less susceptible to antibiotic therapy.

Outside of the phenotypic defense against antibiotics that biofilms provide, bacteria have a number of additional defense mechanisms against an antibiotic threat. Chief amongst these are genotypic responses that render bacteria multi-drug resistant. An example of this phenomenon includes resistance to β-lactam antibiotics that have arisen as a result of mutation, selection, and horizontal gene transfer.[4] The rise of drug resistant bacteria, coupled with the diminished interest in antibiotic research and development from pharmaceutical companies poses a very real threat to modern health care. This epidemic has become so serious that the Infectious Disease Society of American has recently issued a call to action from the medical community.[5] 2-Aminoimidazole (2-AI) derivatives have recently been synthesized in our lab and have been shown to be effective toward biofilm inhibition and dispersion.[6] The 2-aminoimidazole functionality is essentially a guanidine mimetic with a highly modulated pKa and is found in numerous marine natural products. Prototypical examples in this class include the natural products such as ageleferin and oroidin, isolated from the sponge *Agelas conifera* (Figure 1).[7] Given the high efficacy of the 2-AI moiety toward modulating bacterial behavior, it was posited that these compounds might be able to be utilized as adjuvants to existing antimicrobial drugs such as β-Lactams by resensitizing the bacteria to the effects of the antibiotic.

2-Aminoimidazole/triazole (2-AIT) compounds have been previously investigated in our lab and have proved to be very active towards inhibiting and dispersing biofilms of a number of strains of pathogenic bacteria. We recently documented that compound **3** also possesses the ability to lower the MIC of oxacillin against an Iberon clone of MRSA (ATCC BAA-44) four fold.[8] Previous studies in our group have focused on synthetic approaches to generate analogues of the 2-AI tail region 'tail' region of 2-AIT compound and the biological impact these modifications have upon biofilm inhibition and dispersion.[6e, 9] The next logical step in our medicinal chemistry program is to develop synthetic approaches to access defined substitution patterns on the 2-aminoimidazole (2-AI) head region while maintaining the triazole containing tail intact (figure 2), and studying the impact that these substitution patterns have upon biofilm inhibition/dispersion, suppression of antibiotic resistance in multi-drug resistant bacterial strains, and the inherent microbicidal activity of the 2-AI compounds.[6e, 9a, 10] Herein we report a versatile synthetic approach to 1,4 substituted 2-AITs, the use of this approach to the assembly of a pilot library of 2-AIT analogues, and the biological activity of this library against methicillin resistant staphylococcus aureus (MRSA) and multi-drug resistant *Acinetobacter baumannii* (MDRAB).

2. Results and Discussion

2.1. Chemistry

It was initially believed that the synthesis of the 1, 4-substituted 2-AIT could be accomplished by cyclization of an N-substituted amino ketone with cyanamide, as similar experiments in our lab have produced 4,5-substituted 2-AIs from unsubstituted α-amino ketones.[6f] We envisioned the relatively unstable intermediary amino ketone could be synthesized from the nucleophilic ring opening of an epoxide with a primary amine(Figure 3).

The synthesis of epoxide **10** began by isomerizing internal alkyne **5** via the alkyne "zipper" reaction to give terminal alkyne **6** (Scheme 1).[11] An azide-alkyne Huisgen cycloaddition ("click reaction") was then performed on terminal alkyne **6** and azide **7** to provide alcohol **8** in quantitiative yield.[12] The alcohol was then subjected to a combined Swern/Wittig protocol, to deliver alkene **9** in 66% yield over 2 steps.[13] The alkene was then selextively epoxidized with m-chloroperoxybenzoic acid, to yield the key epoxide intermediate **10** in an 80% yield.

We initially hoped to directly open the epoxide with a primary amine substrate at the less hindered carbon that following oxidation of the resultant alcohol would yield α-amino ketone **11**. Pyrimidine and aniline were initially chosen as nucleophiles to open the epoxide, but in each attempt there was no reaction, even upon heating to 60 ^{o}C (Table 1). It was thought that a more nucleophillic primary amine would better serve to open the epoxide. Product was formed upon reaction of the epoxide with 8 equivalents of decylamine, in a DMSO:H_2O solvent system at 60

^{o}C Although this procedure delivered the amino alcohol in a 55% yield, the reactants suffered from poor solubility in the DMSO:H_2O solution and the product contained a considerable amount of impurites that persisted even after multiple attempts at purification. The amino alcohol was also formed upon reaction with undecylamine as the nucleophile; however the use of different conditions (DMF, Montmorillonite K10, 60 ^{o}C) chosen to improve solubility still led to impurites that could not be separated from the product even after multiple attempts at purification. Given the inability to separate the amino alcohol from the impurities associated with the reaction, we subjected each mixture to the Swern oxidation, in an attempt to produce the amino ketone; however in each case produced no product. Smaller molecular weight primary amines, hexyl- and heptyl-, as well as benzylamine, were also used as nucleophiles, under similar conditions, and in every case no product was obtained.

Given the failure of this route to generate the target substitution pattern, we explored the use of a reductive aza-wittig reaction.[14] Secondary amines have been prepared from azides previously by use of this one-pot method by use of a Staudinger reduction followed by imine formation with the corresponding aldehyde, and *in situ* reduction of the crude imine. This was explored by opening epoxide **10** with sodium azide under reflux, yielding the azido-alcohol **12** in 87% yield (Scheme 2). The oxidation of the alcohol was initially attempted with IBX as well as DMP; however neither oxidant produced the corresponding azido amine. The Swern oxidation was found to be an ideal oxidation, producing azido ketone **13** in 94% yield. Next we wanted to perform an aza-wittig reaction on the azido group, followed by a direct reduction of the intermediate imine functionality. This method would be an effective way to produce alkyl derivatives at the N1 position on the 2-AI ring. The azide was reduced with PPh_3 and in the presence of an excess of a corresponding aldehyde, imine formation was observed after two hours in refluxing toluene, however the imine could not be isolated, and all efforts towards *in situ* imine reduction using $Na(CN)BH_3$ and acetic acid proved fruitless, as decomposition was observed in each case.

With the failure of this second route to access the N-substituted amino-ketone intermediate, it became necessary to explore a third route toward synthesizing the key intermediate **11**. Recently, Sorrell and coworkers observed that the reaction of an α-bromo ketone with a primary amine produced the desired N-substituted α-amino ketone, which in their hands was followed by direct reaction with formamide to yield 1,4 substituted imidazoles.[15] Based upon this precedent, it was reasoned that using cyanamide rather than formamide would yield the desired 1,4 substituted 2-AI, which necessitated to synthesis of the α-bromo ketone **15** (Scheme 3). This was accomplished by performing a Jones oxidation on the previously synthesized triazole-alcohol **8**, producing carboxylic acid **14** in 70% yield after purifaction. The carboxylic acid was then reacted with oxalyl chloride producing the intermediate acid-chloride, followed by treatment with diazomethane, which after treatment with HBr to yield α-Bromo ketone **14** in 84% yield over the three steps.

Numerous attempts to react α-bromo ketone **15** with a variety of primary amines failed to provide the desired amino ketone. Upon monitoring the reaction by ^{1}H NMR, it was observed that the bromide displacement was rapid, giving almost full conversion to the amino ketone within 10 minutes evidenced by the shift in the methylene singlets from the starting material to the product (-Δppm 0.4). It was observed that the amino-ketone singlet converted into a multiplet after one hour, corresponding to an undesired side product (most likely self condensation dimer). Based upon this observation, we developed a one-pot tandem procedure for the bromide displacement and subsequent cyanamide cyclization to deliver the target 2-AI derivatives (Scheme 4). This was accomplished by reacting the α-bromo ketone **15** with 0.95 equivalents of the primary amine for 30 minutes in ethanol that was followed by addition of cyanamide. The pH of the reaction was then adjusted to 4.3 by adition of 0.1N HCl_{aq} and the resulting solution was refluxed for three hours to deliver the targeted 1,4 substituted 2AITs as their free bases after chromatography with CH_2Cl_2/MeOH saturated with NH_3. Treatment with HCl provided the final HCl salts for biological testing in yields up to 36% over the 3 steps (Scheme 4, table 2).

2.2. MRSA and MDRAB biofilm modulation

The 2-aminoimidazole moiety has been observed in nature to possess antibiofouling properties[16] and has provided a priveledged scaffold for the development of anti-biofilm compounds.[6a, 6b, 6e, 6f, 9b, 10, 17] Most these 2-AI compounds appear to effect biofilms at both the beginning and final stages of a biofilm development cycle, leading to compounds that both inhibit and disperse bacterial biofilms. Therefore we were initially interested in testing the effect that the 1,4-substitution pattern has upon the 2-AITs ability to inhibit of biofilm formation, as well as induce dispersion of preformed biofilms.

As 1,4 di-substituted 2-AITs have previously not been investigated for antibiofilm activity, we proceeded to evaluate their ability to inhibit biofilm formation by a representative Gram-positive bacterial strain, MRSA (ATCC BAA-44), and a representative Gram-negative bacterial strain, MDRAB (ATCC BAA-1605) We also assesed their ability to disperse preexisting biofilms. Here, we define the 2-AIT concentration required to inhibit 50% of biofilm growth, relative to an untreated control, as the IC_{50} value, while the concentration required to disperse 50% of a preformed biofilm is defined as the EC_{50} value. Finally, we also assayed each compound for their inherent antibiotic activity by determining the MIC of each compound under CLSI conditions using the microdilution protocol.

The ability of each compound to inhibit biofilm development was assessed using the crystal violet reporter assay.[18] The most potent MRSA biofilm inhibitor of the initial library, **4h**, contained an n-pentyl substitutent (Table 3). Compound **4h** exhibited an IC_{50} of 4.14μM against MRSA, which was slightly lower than the MIC of our lead compound **3**, and was also shown to act in a non-microbicidal fashion according to growth curve analysis. In an attempt to

increase activity, analogues of **4h** were synthesized in which the alkyl chain was either shortened to *n*-butyl, 4g, and lengthened to *n*-hexyl, **4i**. However in both cases activity decreased as we observed IC_{50}'s of 10.8, and 6.2 μM respectively. The benzyl substituted 2-AI, **4k**, gave a promising IC_{50} of 7.16 μM. Again, we attempted to augment activity by synthesizing analogues in which we inserted a methylene unit, **4l**, or removed a methylene unit, **4j**; however both analogues had dimineshed anti-biofilm activity, returning IC_{50} values of 25.1 and 9.9 μM respectively. The cyclopentyl derivative, **4b**, gave an IC_{50} of 9.01 μM, but again as in the previous cases, decreasing or increasing the ring size to a cyclopropy, **4a**, or cyclohexyl, **4c**, delivered compounds with decreased activity (IC_{50}'s of >100 μM and 18.7 μM respectively).

We were also interested determining the activity of our 2-AITs against a multi-drug resistant Gram-negative bacterium. To this end, we investigated MDRAB. *A. baumannii* is nosocomial pathogen that survives in hospital settings based, in part, by its ability to form robust biofilms.[19] The antimicrobial activity and biofilm inhibition activity were determined for all compounds (table 3). When compared to MRSA, these compounds were less active towards MDRAB displaying much higher MIC and IC_{50} values. The leading biofilm inhibitor was hexyl derivative **4i**, with an IC_{50} of 26.2 μM, followed closely by the n-pentyl **4h**, cyclopentyl **4b**, and benzyl derivative **4k**, with IC_{50} values of 31.4, 33.7, and 33.9 μM respectively. Furthermore, four of the compounds, **4a**, **4c**, **4d** and **4f**, showed sharp drops in inhibition over a very narrow range in concentrations, usually indicative of toxic mechanisms.

Finally, we were interested in the dispersion potential of our 2-AITs with a preformed biofilm. Dispersion of a preformed biofilm is a very desirable property as compounds which simply inhibit biofilm formation will mostly likely find utility as prophylactic agents, while compounds that disperse pre-formed biofilms have the potential to treat an established disease state. The 5 most potent biofilm inhibitors, **4b**, **4h**, **4i**, **4k** and **4l** were selected for analysis, and their EC_{50}s were determined against MRSA. All compounds exhibited similar biofilm dispersion activity against MRSA, and returned EC_{50} values of 33.0 – 45.1 μM (Table 4).

2.3. Resensitization of MRSA

Due to the growing problem of multi-drug resistant bacteria, our group has been exploring the ability of suitably derived 2-AIs to suppress antibiotic resistance in multi-drug resistant bacteria.[17d, 20] To this end, we were interested in testing the potential of our 1,4 substituted 2-AITs to resensitize MDR bacteria towards oxacillin, since 2-AIT **3** has been documented to reduce the MIC of oxacillin four-fold at 25% of its MIC (25 μM).[8] To study the resensitization effects, the minimum inhibitory concentration (MIC) of each 2-AIT against MRSA (ATCC BAA-44) was initially determined by using a microdilution protocol. To probe suppression of antibiotic resistance, the MIC of oxacillin was determined in the presence of 25% the MIC of each 2-AIT compound. In our experience, 2-AI compounds themselves at 25% their MIC value display limited toxicity to bacteria. Mueller Hinton Broth was inoculated with BAA 44, and oxacillin was serially diluted in both the inoculated media alone to serve as a control, as

well as inoculated media supplemented with 2-AITs. The MICs of the oxacillin in the presence of the 2-AITs were compared with that of oxacillin alone, and a fold-reduction was noted by dividing the oxacillin MIC by the MIC of oxacillin with the 2-AITs (Table 5). The most active compound was **4e**, which has an isobutyl substituent at the 1' nitrogen. Compound **4e** elicited a four-fold reduction in the MIC of oxacillin. Unfortunately no other compounds tested were able to match the resensitization ability of the lead compound **4e** or the parent compound **3**.

Finally, the checkerboard assay was used to determine if there was any synergism between the 2-AIT compounds and oxacillin. [21] The assay is performed by serially diluting one antibiotic vertically down a 96-well plate, followed by the second antibiotic horizontally across the plate. The fractal inhibitory concentration (FIC) is then calculated by dividing the MIC of each antibiotic in the combinations, by that of the antibiotic alone. An FIC index (ΣFIC) is than determined for each well by adding each of the individual antibiotic FICs corresponding to that well. A ΣFIC of ≤ 0.5 is indicative of synergistic effects between the two antibiotics. The lead compound in our library gave a ΣFIC of 0.5 suggesting synergism between the compound and oxacillin. However all other 1, 4 substituted 2-AIT tested gave a ΣFIC was higher than 0.5 (Table 5), further confirming the lack of synergy between the 1,4-substituted 2-AITs and oxacillin.

3. Conclusion

We have successfully developed an efficient approach to the synthesis of 1,4-substituted-2-aminoimidazole/triazole conjugates via a key one-pot N-alkylation/cyanimide cyclization procedure, and have employed this strategy to assemble a pilot library of 2-AIT derivatives. This synthesis requires the use of no protecting groups, and can be further used for the construction of a library of general 1,4-substituted 2-AIs. The compounds were found to be active biofilm inhibitors, with the lead compound being *n*-pentyl derivative **4h**. Compound **4h**, as well as **4g** (*n*-butyl) and **4f** (allyl) inhibited biofilm formation through a non-microbicidal mechanism. Compounds **4b**, **4i**, **4k**, and **4l** were also active; however growth curve analysis showed that these compounds acted in a microbicidal fashion at their IC_{50} concentration. Combination studies with these compounds demonstrated that certain 2-AIT analogues suppressed oxacillin resistance in the MRSA strain, with the lead compound, **4e**, effecting a four-fold reduction in the MIC of oxacillin, as well as a ΣFIC of 0.500 indicating a synergistic behaviour between compound **4e** and oxacillin. Given the potential of the 2-AIT scaffold to serve a platform to design molecules that both inhibit/disperse biofilms and suppress antibiotic resistance in MDR bacteria, we are currently exploring the potential of lead 2-AIT derivatives and other 2-AI compounds in animal models of MDR bacterial infection. These studies will be reported in due course.

4. Experimental Section

4.1. Chemistry

All reagents used for chemical synthesis were purchased from commercially available sources, and required no further purification. Chromatography was performed with 60 Å mesh standard grade silica gel from Sorbtech. Infrared spectra were obtained on an FT/IR-4100 spectrophotometer (ν max in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/Vis spectrophotometer (λ_{max} in nm). NMR solvents were obtained from Cambridge Isotope Labs. ^{1}H NMR (400MHz and 300 MHz) and ^{13}C NMR (100 MHz) spectra were recorded at 25^{o}C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to their respective solvent. Coupling constants (J) are in Hertz (Hz). Abbreviations are as followed: s = singlet, d = doublet, t = triplet, dt = doublet of triplets, brs = broad singlet, and m = multiplet. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. MRSA (ATCC # BAA-44), and MDRAB (ATCC # BAA-1605) were obtained from the American Type Culture Collection. Mechanically defribulated sheep blood (DSB100) was obtained from Hemostat Labs. Oxacillin sodium salt was purchased from Fluka.

Synthesis of N-(2-(4-(6-hydroxyhexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (8) 1-Octyn-ol (10 g, 79.2 mmol) was dissolved in 150 mL of tBuOH/H_2O/DCM(2/2/1), and at room temperature was added N-(2-azidoethyl)-4-pentylbenzamide (xx)(22.7 g, 87.16 mmol), $CuSO_4$ (1.89 g, 12 mmol) and sodium ascorbate (6.27 g, 31.7 mmol), and the reaction was stirred for 16 hours. Reaction was quenched with water and extracted with a 10% MeOH in DCM solution 2 times, and once with DCM. The organic layers were combined and washed with brine, dried with magnesium sulfate, filtered, and concentrated *in vacuo*. Crude mixture was purified by flash chromatography (2.5% to 10% gradient MeOH/DCM) to provide N-(2-(4-(6-hydroxyhexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (30.154 g, 99% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, *J* = 8.4 Hz, 2H), 7.31 (s, 2H), 7.18 (d, *J* = 8 Hz, 2H), 4.54 (t, *J* = 5.8 Hz, 2H), 3.91 (dt, *J* = 5.6, 5.6, 2H), 3.58 (t, J = 6.4 Hz, 2H), 2.66-2.58 (m, 4H), 2.32(brs, 1H), 1.62-1.52(m, 4H), 1.52-1.47(m, 2H), 1.33-1.26(m, 8H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.2, 148.3, 147.4, 131.4, 128.8, 127.4, 122.2, 62.8, 49.6, 40.1, 36.0, 32.7, 31.6, 31.1, 29.4, 28.9, 25.5, 25.5, 22.7, 14.2; IR ($CDCl_3$) 3356, 3297, 2927, 2853, 2359, 2338, 1641, 1540, 1303, 1055; UV (λ_{max} nm) 238; observed melting point: 94^{o}C; HRMS (ESI+) m/z 387.2746 [$(M+H)^+$; calculated mass for $C_{22}H_{34}N_4O_2^+$: 387.2755 amu].

N-(2-(4-(hept-6-enyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (9). To a solution of oxalyl chloride (13.8 mL, 156 mmol) in 340 mL of DCM at -78^{o}C was added DMSO (27.7 mL, 390 mmol) dropwise. After 10 minutes, N-(2-(4-(6-hydroxyhexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was added dropwise over 10 minutes. This solution was then stirred for 30 minutes, until triethyl amine (75.7 mL, 546 mmol) was added dropwise, and stirring continued. After 30 minutes the cold bath was removed and the solution was allowed to warm to room temperature. Reaction was diluted with DCM then washed with water (3x500mL), and brine (1x300mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in*

vacuo, and the resultant N-(2-(4-(6-oxohexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was kept under high vacuum until subsequent use. To a slight slurry of methyltriphenylphosphonium bromide(30 g, 78 mmol) in 390 mL of toluene at -30^{o}C was added 0.91 molar potassium bis(trimethyl)silyl amide(214.3 mL, 195 mmol) dropwise over 30 minutes, and the solution was allowed to stir for 30 minutes at room temperature. The reaction vessel was cooled back down to -30^{o}C whereupon N-(2-(4-(6-oxohexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was added dropwise dissolved in 100 mL of THF. Cold bath was removed after addition. After 20 minutes 300 mL of brine was poured into the reaction vessel, and the resultant mixture was extracted with DCM (3 x 300 mL), and the organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo.* Crude mixture was purified by flash chromatography (50%-100% Ethyl Acetate in Hexanes) to provide N-(2-(4-(hept-6-enyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (19.67 g, 66%), as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85 (t, *J* = 5.6 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.26 (s, 1H), 7.132 (d, *J* = 8 Hz, 2H), 5.76-5.66 (m, 1H), 4.93-4.85 (m, 2H), 4.50 (t, *J* = 5.4 Hz, 2H), 3.87 (dt, *J* = 5.2, 6 Hz, 2H), 2.58-2.52 (m, 4H), 1.97-1.92 (m, 2H), 1.59-1.51 (m, 4H), 1.34-1.21 (m, 8H), 0.83 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.3, 148.2, 147.2, 139.0, 131.5, 128.7, 127.5, 122.1,114.6, 49.5, 40.3, 36.0, 33.8, 31.6, 31.1, 29.5, 28.9, 28.8, 25.7, 22.7, 14.2; IR ($CDCl_3$) 3308, 3144, 3078, 2959, 2927, 2854, 1647, 1556, 1506, 1434,1333, 1304,1053, 909; UV (λ_{max} nm) 238; observed melting point: 87^{o}C; HRMS (ESI+) m/z 383.2802 [$(M+H)^+$; calculated mass for $C_{22}H_{34}N_4O_2^+$: 383.2805].

N-(2-(4-(5-(oxiran-2-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (10). To a solution of N-(2-(4-(hept-6-enyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (16.7 g, 43.6 mmol) dissolved in 300 mL of DCM at 0^{o}C was added mCPBA(24.5 g, 109 mmol). The reaction vessel was then removed from cold bath and extracted with DCM (3x200 mL), and organic layers were combined and washed with aq. $NaHCO_3$(2x100 mL), and brine (1x100 mL). The solution was dried with magnesium sulfate, filtered and concentrated *in vacuo*. Crude mixture was purified by flash chromatography (100% EtOAc) to afford N-(2-(4-(5-(oxiran-2-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (13.92 g, 80%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66 (d, *J* = 8 Hz, 2H), 7.30 (s, 1H), 7.22 (d, *J*= 8Hz, 2H), 6.81 (brs, 1H), 4.56 (t, *J* = 6 Hz, 2H), 3.96 (dt, *J* = 6 Hz, 5.6 Hz, 2H) 2.88 (m, 1H), 2.74-2.68 (m, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 2.45-2.43 (m, 1H), 1.69-1.28 (m, 15H), 0.88 (t, *J* = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.1, 148.3, 147.4, 131.4, 128.8, 127.3, 122.1, 52.5,49.5, 47.2, 40.1, 36.0, 32.5, 31.6, 31.1, 29.5, 29.1, 25.9, 25.6, 22.7, 14.2; IR ($CDCl_3$) 3313, 3295, 3146, 2928, 2851, 2365, 2330, 1643, 1549, 1301, 1058; (λ_{max} nm) 238; observed melting point: 90^{o}C; HRMS (ESI+) m/z 399.2751 [$(M+H)^+$; calculated mass for $C_{22}H_{34}N_4O_2^+$: 399.2755].

N-(2-(4-(7-azido-6-hydroxyheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (11). N-(2-(4-(5-(oxiran-2-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide(1.17 g, 2.94 mmol) was dissolved in 30 mL of 2-methoxyethanol/water(8:1). To this solution was added sodium azide (0.5734 g, 8.82 mmol) and ammonium chloride (0.173 g, 3.23 mmol). The reaction was

stirred at reflux (150^{o}C) for 3 hours, at which point the reaction was allowed to cool back down to room temperature, and then quenched with water and diluted with EtOAc. Resultant mixture was washed with water (3x50mL) and brine (1x 50mL) and then dried with magnesium sulfate, filtered, concentrated *in vacuo*, and placed under high vacuum overnight. Product obtained was pure N-(2-(4-(7-azido-6-hydroxyheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (1.13g, 87%) as a white solid, and needed no further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.45 (s, 1H), 7.32 (s, 1H), 7.16 (d, *J* = 8.4 Hz, 2H), 4.52 (t, *J* = 5.4 Hz, 2H), 3.88 (dt, *J* = 6 Hz, 5.2 Hz, 2H) 3.73-3.70 (m, 1H), 3.27-3.16 (m, 3H), 2.63-2.56 (m, 4H), 1.60-1.54 (m, 4H), 1.42-1.24 (m, 10H), 0.85 (t, *J* = 7Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.3, 147.5, 132.3, 132.2, 128.8, 127.4, 122.2, 70.7, 57.2, 49.5, 40.1, 36.0, 34.4, 31.6, 31.1, 29.3, 28.9, 25.4, 25.2, 22.7, 14.2; IR ($CDCl_3$) 3351, 2928, 2855, 2102, 1643, 1540, 1504, 1437, 1302, 1054; (λ_{max} nm) 238; observed melting point: 84^{o}C; HRMS (ESI+) m/z 442.2922 [$(M+H)^{+}$; calculated mass for $C_{23}H_{36}N_7O_2^{+}$: 442.2930].

N-(2-(4-(7-azido-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (12). To a solution of oxalyl chloride(.31 mL mL, 3.5 mmol) in 7 mL of DCM at -78^{o}C was added DMSO (.062 mL, 8.73 mmol) dropwise. After 10 minutes, N-(2-(4-(7-azido-6-hydroxyheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide(0.77 mg, 1.75 mmol) was added dropwise over 10 minutes. This solution was then stirred for 30 minutes, until triethyl amine (1.7 mL, 12.25 mmol) was added dropwise, and stirring continued. After 30 minutes the cold bath was removed and the solution was allowed to warm to room temperature. Reaction was diluted with DCM then washed with water (3x30mL), and brine (1x30mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*, affording pure N-(2-(4-(7-azido-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (0.719 g, 94%) as a white solid . ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, *J* = 8 Hz, 2H), 7.47 (s, 1H), 7.32 (s, 1H), 7.14 (d, *J* = 8Hz, 2H), 4.52 (t, *J* = 5.8 Hz, 2 H), 3.86 (dt, *J* = 6 Hz, 6 Hz, 2H), 3.08-3.01 (m, 2H), 2.62-2.54 (m, 4H), 2.36 (t, *J* = 7.4, 2H), 1.60-1.51 (m, 4H), 1.33 (t, *J* = 7.4 Hz, 2H), 1.281-1.20 (m, 6H), 0.824 (t, *J* = 7Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.7, 168.1, 147.3, 132.3, 132.2, 129.0, 127.4, 122.2, 57.5, 49.5, 46.1, 39.9, 36.0, 31.6, 31.1, 29.1, 28.5, 25.4, 23.1, 22.6, 14.2; IR ($CDCl_3$) 3307, 3121, 3070, 2930, 2854, 2738, 2677, 2491, 2104, 1717, 1638, 1541, 1291; (λ_{max} nm) 238; observed melting point: 84^{o}C; HRMS (ESI+) m/z 440.2763 [$(M+H)^{+}$; calculated mass for $C_{23}H_{34}N_7O_2^{+}$: 440.2774].

6-(1-(2-(4-pentylbenzamido)ethyl)-1H-1,2,3-triazol-4-yl)hexanoic acid (14). To a stirred solution of N-(2-(4-(6-hydroxyhexyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide(8.94 g, 23.13 mmol) dissolved in 30 mL of acetone at 0^{o}C was added freshly made 2 mM Jones reagent(20mL) dropwise until titrated solution persisted green color indication oxidation had completed. Reaction mixture then allowed to stir at room temperature for an additional 50 minutes, at which point 75 mL of water was added. Aqueous layer was extracted with ethyl ether (2x60 mL) , and the ether layers were combined, and back extracted with 1M aq. NaOH (2x60 mL). The combined aqueous layers were combined and acidified with concentrated HCl

until pH indicated a pH of 1-2, and aqueous layer was extracted with ether (2x60mL). Ether extracts were combined and washed with brine, dried with magnesium sulfate, filtered, and concentrated *in vacuo*. Product afforded was pure 6-(1-(2-(4-pentylbenzamido)ethyl)-1H-1,2,3-triazol-4-yl)hexanoic acid (6.436 g, 70 %) . ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, *J* = 8 Hz, 2H), 7.37 (s, 1H), 7.31 (brs, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 4.55 (t, *J* = 5.6 Hz, 2H), 3.91 (dt, *J* = 5.6 Hz, 5.6 Hz, 2H), 2.66 (t, *J* = 7.4 Hz, 4H), 2.61 (t, *J* = 8 Hz, 2H), 1.67-1.55 (m, 6H), 1.38-1.25 (m, 6H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.0, 168.4, 148.0, 147.6, 131.2, 128.8, 127.4, 122.5, 49.7, 40.1, 36.0, 34.0, 31.6, 31.1, 28.9, 28.4, 25.2, 24.5, 22.7, 14.2; IR ($CDCl_3$) 3735, 3435, 2935, 2849, 2362, 2008, 1637, 1426, 1057; (λ_{max} nm) 238; observed melting point: 100^{o}C; HRMS (ESI+) m/z 401.2547 [$(M+H)^+$; calculated mass for $C_{22}H_{34}N_4O_2^+$: 401.2550].

N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (15). To a solution of 6-(1-(2-(4-pentylbenzamido)ethyl)-1H-1,2,3-triazol-4-yl)hexanoic acid (2.8 g, 7 mmol) dissolved in 40 mL DCM, to which was added 5-10 drops of DMF at 0^{o}C. At 0^{o}C was added oxalyl chloride (2 mL, 23 mmol) dropwise. The reaction continued stirring for 15 minutes at 0^{o}C, and then allowed to warm to room temperature, and stirring continued for an additional hour. The reaction was then concentrated *in vacuo*, and placed under high vacuum for 2 hours. The crude mixture was then dissolved in 20 mL of DCM, and placed in an ice bath. At 0^{o}C the crude mixture dissolved in DCM was added dropwise to a freshly made diazomethane (35 mmol) in 80 mL of ethyl ether. The reaction was allowed to stir for 1 hour. At 0^{o}C was added 4 mL of HBr to the mixture, and the reaction was stirred for an additional 30 minutes. Reaction was quenched with 100 mL of aqueous $NaHCO_3$, and stirred for 10 minutes. 200 additional mL was added to the reaction flask, and reaction was washed with aqueous $NaHCO_3$ (3x100) and brine (1x100), then organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo* to afford pure N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (2.8 g, 84%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, *J* = 8.1 Hz, 2H), 7.32 (s, 1H), 7.20 (d, *J* = 8.1 Hz, 2H), 7.08 (s, 1H), 4.56 (t, *J* = 5.4 Hz, 2H), 3.94 (dt, *J* = 5.6 Hz, 5.6 Hz, 2H), 3.85 (s, 2H), 2.70-2.59 (m, 6H), 1.67-1.57 (m, 6H), 1.34-1.29(m, 6H), 0.87 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.3, 168.2, 147.3, 131.4, 128.7, 127.4, 122.2, 60.4, 49.5, 48.5, 40.2, 36.0, 34.3, 31.6, 31.1, 28.7, 28.5, 25.4, 24.8, 22.7, 14.2; IR ($CDCl_3$) 3311, 3120, 3070, 2929, 2854, 1719, 1637, 1540, 1295, 1051; (λ_{max} nm) 238; observed melting point: 89^{o}C; HRMS (ESI+) m/z 477.1854 [$(M+H)^+$; calculated mass for $C_{23}H_{33}BrN_4O_2^+$: 477.1860 amu].

General procedure for synthesis of 1,4-2 AIs:

N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide (100 mg, 0.21 mmol) was dissolved in 95% ethanol. At room temperature R-NH_2 (20 mmol, 0.95 eq) was added dropwise to solution, and reaction was allowed to stir for 30 minutes. Cyanamide (0.264 g, 6.3 mmol) was added to the reaction vessel, and the pH was adjust to 4.3, using .1M HCl. Reaction was refluxed at 95^{o}C for 3 hours, then allowed to cool to room temperature, and

concentrated *in vacuo*. Crude mixture was purified by flash chromatography (1%-7% MeOH sat. with NH_3 in DCM) to afford the free base. Free base put under high vacuum for 3 hours to remove any excess NH_3, then diluted with DCM, and treated with 5 drops of concentrated HCl. Mixture concentrated *in vacuo*, and then placed under high vacuum overnight to afford pure product as the HCl salt.

N-(2-(4-(5-(2-amino-1-cyclopropyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4a). Following the general procedure for they synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with cyclopropylamine to provide N-(2-(4-(5-(2-amino-1-cyclopropyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (17 mg, 16%) as a yellow oil.; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.61(s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H), 6.54 (s,1H), 4.84 (t, *J* = 5.3 Hz, 2H), 3.94 (t, *J* = 5.0 Hz, 2H), 3.08-3.02 (m, 1H), 2.88 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.43 (t, *J* = 7.5 Hz, 2H), 1.78-1.56 (m, 5H), 1.45-1.30 (m, 6H), 1.11 (dt, *J* = 7.8 Hz, 6.6 Hz, 2H), 0.97-0.87 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 144.3, 131.0, 128.5, 127.6, 127.2, 126.5, 111.8, 53.2, 39.2, 35.5, 31.3, 31.0. 27.8, 27.6, 27.5, 25.9, 24.0, 22.7, 22.4, 13.2, 6.0; IR ($CDCl_3$) 3326, 2928, 2855, 2240, 1686, 1541, 1457, 1056, 975, 817; (λ_{max} nm) 238; HRMS (ESI+) m/z 478.3290 [$(M+H)^+$; calculated mass for $C_{27}H_{40}ClN_7O^+$: 478.3289 amu].

N-(2-(4-(5-(2-amino-1-cyclopentyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4b). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with cyclopentylamine to provide N-(2-(4-(5-(2-amino-1-cyclopentyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (18 mg, 16%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.61(s, 1H), 7.69 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* =8.4 Hz, 2H), 6.69 (s, 1H), 4.85 (t, *J* = 5.2 Hz, 2 H), 4.44-4.41(m, 1H), 3.94 (t, *J* = 5.2 Hz, 2H), 2.89 (t, *J* = 5.2 Hz, 2H), 2.63 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 2.14 (m, 2H), 1.87-1.61 (m, 10H), 1.42-1.32 (m, 8H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 145.9, 144.3, 131.0, 128.5, 127.6, 127.3, 108.5, 56.2, 53.2, 39.2, 35.5, 31.5, 31.4, 31.0, 27.9, 27.6, 24.2, 23.5, 22.7, 22.4, 13.2; IR ($CDCl_3$) 3294, 3140, 2930, 2859, 1655, 1539, 1503, 1455, 1302, 1057, 856; (λ_{max} nm) 238; HRMS (ESI+) m/z 506.3592 [$(M+H)^+$; calculated mass for $C_{29}H_{44}ClN_7O^+$: 506.3607 amu].

N-(2-(4-(5-(2-amino-1-benzyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4k). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with benzylamine to provide N-(2-(4-(5-(2-amino-1-benzyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (27 mg, 23%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.62(s, 1H), 7.69 (d, *J* = 8 Hz, 2H), 7.41-7.27 (m, 5H), 7.24 (d, *J* = 8 Hz, 2H), 6.56 (s, 1H), 5.04 (s, 2H), 4.85 (t, *J* = 5.2 Hz, 2H), 3.94 (t, *J*

= 5.2 Hz, 2H), 2.87 (t, *J* = 7.6 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 1.75-1.56 (m, 6H), 1.43-1.26 (m, 6H), 0.88 (t, *J* = 7 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 146.4, 144.2, 134.9, 131.0, 129.0, 128.5, 128.4, 127.6, 127.5, 127.3, 112.0, 53.2. 39.2, 35.5, 31.3, 31.0, 27.8, 27.5, 27.4, 24.0, 22.7, 22.3, 13.2; IR ($CDCl_3$) 3281, 3128, 2930, 2866, 1663, 1540, 1501, 1455, 1305, 1188, 1054, 975; (λ_{max} nm) 238; HRMS (ESI+) m/z 528.3462 [$(M+H)^+$; calculated mass for $C_{31}H_{41}N_7O^+$: 528.3445 amu].

N-(2-(4-(5-(2-amino-1-hexyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4i). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with hexylamine to provide N-(2-(4-(5-(2-amino-1-hexyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (29 mg, 25%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.24 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 6.59 (s, 1H), 4.74 (t, *J* = 5.2 Hz, 2H), 3.89 (t, *J* = 5.2 Hz, 2H), 3.77 (t, *J* = 7.1 Hz, 2H), 2.80 (t, *J* =6.9 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.44 (t, *J* = 7.5 Hz, 2H), 1.17 (m, 4H), 1.61 (m, 4H), 1.33 (m, 12H), 0.914-0.870 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.47, 146.1, 145.5, 131.2, 128.8, 127.2, 126.1, 112.0, 52.0, 45.1, 39.45, 35.5, 31.4, 31.0, 28.7, 28.1, 28.0, 27.6, 25.9, 24.1, 23.5, 22.4, 13.2; IR ($CDCl_3$) 3112, 2929, 2857, 2361, 1660, 1540, 1503, 1439, 1053, 973; (λ_{max} nm) 238; HRMS (ESI+) m/z 522.3906 [$(M+H)^+$; calculated mass for $C_{30}H_{47}N_7O^+$: 522.3915 amu].

N-(2-(4-(5-(2-amino-1-phenethyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4l). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with phenethylamine to provide N-(2-(4-(5-(2-amino-1-phenethyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (12 mg, 10%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.31 (s, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.30-7.15 (m, 7H), 6.39 (s, 1H), 4.75 (t, *J* = 5.7 Hz, 2H), 4.04 (t, *J* = 7.1 Hz, 2H), 3.90 (t, *J* = 5.4 Hz, 2H), 3.02 (t, *J* = 6.9 Hz, 2H), 2.8 (t, *J* = 7.5 Hz, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 2.39 (t, *J* = 7.5 Hz, 2H), 1.72-1.53 (m, 6H), 1.34-1.29 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3-OD, 100 MHz) δ 169.3, 147.5, 137.2, 131.2, 128.8, 128.6, 128.5, 127.2, 126.9, 125.6, 112.0, 51.7. 46.2, 39.4, 35.5, 34.5, 31.4, 31.0, 29.5, 28.1, 27.8, 27.5, 23.9, 23.5, 22.3, 13.2; IR ($CDCl_3$) 3121, 2927, 2855, 1660, 1540, 1499, 1454, 1180, 1056, 973; (λ_{max} nm) 238; HRMS (ESI+) m/z 542.3600 [$(M+H)^+$; calculated mass for $C_{32}H_{43}N_7O^+$: 542.3602 amu].

N-(2-(4-(5-(2-amino-1-butyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4g). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with butylamine to provide N-(2-(4-(5-(2-amino-1-butyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (40 mg, 36%) as a yellow oil. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.59 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.26

(d, *J* = 8 Hz, 2H), 6.61 (s, 1H), 4.83 (t, *J* = 5.6 Hz, 2H), 3.94 (t, *J* = 5.6 Hz, 2H), 3.78 (t, *J* = 7.4 Hz, 2H), 2.88 (t, *J* = 7.8 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.8, 2H), 1.76-1.69 (m, 4H), 1.62 (m, 4H), 1.43-1.28 (m, 8H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 162.3, 147.5, 146.1, 144.8, 131.2, 128.5, 127.3, 127.0, 112.0, 52.7, 44.9, 39.3, 35.5, 31.4, 31.0, 30.8, 27.9, 27.8, 27.6, 24.1, 23.1, 22.4, 19.5, 13.2, 12.8; IR ($CDCl_3$) 3280, 3128, 2931, 2857, 1725, 1661, 1538, 1468, 1376, 1288, 1162, 1058; (λ_{max} nm) 238; HRMS (ESI+) m/z 494.3602 [$(M+H)^+$; calculated mass for $C_{28}H_{44}N_7O^+$: 494.3624 amu].

N-(2-(4-(5-(2-amino-1-isobutyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4e). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with isobutylamine to provide N-(2-(4-(5-(2-amino-1-isobutyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (15 mg, 14%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.42 (s, 1H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.26 (d, 8.1 Hz, 2H), 6.58 (s, 1H), 7.48 (t, *J* = 4.2 Hz, 2H), 3.91 (t, *J* = 5.4 Hz, 2H), 3.60 (d, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.07 (m, 1H), 1.73-1.59 (m, 5H), 1.40-1.28 (m, 6H), 0.95-0.87 (m, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 144.5, 131.1, 128.5, 127.3, 127.2, 127.0, 112.5, 53.1, 51.8, 39.2, 35.5, 31.4, 31.0, 28.4, 27.9, 27.6, 27.5, 24.0, 22.8, 22.4, 18.9, 18.8, 18.6, 13.2; IR ($CDCl_3$) 3274, 3134, 2946, 2931, 2884, 2855, 1678, 1655, 1544, 1456, 1299; (λ_{max} nm) 238; HRMS (ESI+) m/z 494.3589 [$(M+H)^+$; calculated mass for $C_{28}H_{43}N_7O^+$: 494.3602 amu].

N-(2-(4-(5-(2-amino-1-phenyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4j). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with aniline to provide N-(2-(4-(5-(2-amino-1-phenyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (30 mg, 26%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.60 (s, 1H), 7.68 (d, *J* = 6.6 Hz, 2H), 7.66-7.49 (m, 5H), 7.26 (d *J* = 8.4 Hz, 2H), 6.76 (s, 1H), 4.83 (t, *J* = 5.4 Hz, 2H), 3.94 (t, *J* = 5.4 Hz, 2H), 2.90 (t, *J* = 7.5 Hz, 2H), 2.66 (t, *J* = 7.7 Hz, 2H), 2.55 (t, *J* = 7.4 Hz, 2H), 1.79-1.58 (m, 6H), 1.47 (m, 2H), 1.32 (m, 4H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.5, 146.3, 144.7, 134.3, 131.1, 129.8, 128.5, 128.0, 127.3, 127.1, 125.5, 113.2, 52.8, 39.3, 35.5, 31.4, 31.0, 27.9, 27.7, 27.4, 24.0, 22.9, 22.4, 13.2; IR ($CDCl_3$) 3266, 3111, 2930, 2857, 2363, 1658, 1537, 1503, 1456, 1302, 1190, 1052; (λ_{max} nm) 238; HRMS (ESI+) m/z 514.3279 [$(M+H)^+$; calculated mass for $C_{30}H_{39}N_7O^+$: 514.3289 amu].

N-(2-(4-(5-(1-allyl-2-amino-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4f). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with allylamine to provide N-(2-(4-(5-(1-allyl-2-amino-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (27 mg,

25%) as a yellow oil. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.58 (s, 1H), 7.68 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 6.56 (s, 1H), 5.78-5.90 (s, 1H), 5.34-5.29 (s, 1H), 5.19-5.13 (s, 1H), 4.83 (t, *J* = 5.4 Hz, 2H), 4.43 (m, 2H), 3.94 (t, *J* = 5.4 Hz, 2H), 2.88 (t, *J* = 7.6 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 2.47 (t, *J* = 7.4 Hz, 2H), 1.76-1.70 (m, 2H), 1.62 (m, 4H), 1.43-1.39 (m, 2H), 1.33-1.28 (m, 4H), 0.89 (t, *J* = 7 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 144.4, 131.0, 128.5,127.4, 127.2, 117.8, 111.9, 53.1, 47.0, 39.2, 35.5, 31.4, 30.9, 27.8, 27.6, 27.5, 24.0, 22.7, 22.4, 13.2; IR ($CDCl_3$) 3320, 2926, 2855, 1665, 1539, 1503, 1455, 1302, 1160; (λ_{max} nm) 238; HRMS (ESI+) m/z 478.3283 [$(M+H)^+$; calculated mass for $C_{27}H_{40}N_7O^+$: 478.3289 amu].

N-(2-(4-(5-(2-amino-1-pentyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4h). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with pentylamine to provide N-(2-(4-(5-(2-amino-1-pentyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (30 mg, 26%) as a yellow oil. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.50 (s, 1H), 7.67 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* = 8 Hz, 2H), 6.60 (s, 1H), 4.81 (t, *J* = 5.2 Hz, 2H), 3.93 (t, *J* = 5.2 Hz, 2H), 3.77 (t, *J* = 7 Hz, 2H), 2.86 (t, *J* = 7.4 Hz, 2H), 2.45 (t, *J* = 7 Hz, 2H), 1.75-1.70 (m, 4H), 1.61 (m, 4H), 1.42-1.29 (m, 10H), 0.95-0.87 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.6, 146.1, 144.3, 131.0, 128.5, 127.5, 127.2, 111.9, 53.2, 45.0, 39.2, 35.5, 31.3, 30.9, 28.4, 28.4, 27.8, 27.6, 27.5, 24.0, 22.7, 22.3, 22.1, 13.2, 13.1; IR ($CDCl_3$) 3317, 2930, 2857, 2202, 1664, 1539, 1503, 1457, 1377, 1304, 1163, 1084; (λ_{max} nm) 238; HRMS (ESI+) m/z 508.3742 [$(M+H)^+$; calculated mass for $C_{29}H_{45}N_7O^+$: 508.3758 amu].

N-(2-(4-(5-(2-amino-1-cyclohexyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4c). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with cyclohexylamine to provide N-(2-(4-(5-(2-amino-1-cyclohexyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (33 mg, 28%) as a yellow oil. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.62 (s, 1H), 7.69 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 6.73 (s, 1H), 4.85 (t, *J* = 5.2 Hz, 2H), 4.35-4.24 (m, 1H), 3.94 (t, *J* = 5.2 Hz, 2H), 2.89 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 2.02-1.23 (m, 22H), 0.89 (t, *J* = 7 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 147.5, 144.3, 131.2, 131.0, 128.7, 128.5, 127.4, 127.2, 108.4, 54.5, 53.2, 50.4, 39.2, 39.5, 32.0, 31.4, 31.0, 30.8, 27.9, 27.6, 25.1, 24.8, 24.2, 22.7, 22.4, 13.2; IR ($CDCl_3$)3318, 2930, 2859, 2343, 1727, 1689, 1541, 1457, 1338, 1062; HRMS (ESI+) m/z 520.3752 [$(M+H)^+$; calculated mass for $C_{30}H_{45}N_7O^+$: 520.3758 amu].

N-(2-(4-(5-(2-amino-1-isopropyl-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (4d). Following the general procedure for the synthesis of 1,4-subsituted 2-aminoimidazoles, N-(2-(4-(7-bromo-6-oxoheptyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide was reacted with isopropylamine to provide N-(2-(4-(5-(2-amino-1-isopropyl-

1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-pentylbenzamide hydrochloride (36 mg, 33%) as a yellow oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.42 (s, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* =8.4 HZ. 2H), 6.73 (s, 1H), 4.79 (t, *J* = 5.7 Hz, 2H), 4.35-4.30 (m, 1H), 3.92 (t, *J* = 5.3 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 2.64 (t, *J* = 7.4 Hz, 2H), 2.46 (t, *J* = 7.7 Hz, 2H), 1.75-1.67 (m, 3H), 1.66-1.56 (m, 3H), 1.39 (d, *J* = 6.6 Hz, 6H), 1.32-1.29 (m, 6H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 161.8, 147.5, 145.3, 131.2, 128.5, 127.7, 127.2, 125.8,107.7, 51.5, 39. 5, 35.5, 31.4, 30.9, 28.1, 28.0, 27.7, 23.6, 22.7, 20.7, 13.2; IR ($CDCl_3$) 3344, 2937, 2860, 2542, 1696, 1652, 1546, 1457, 1335, 1159, 1056; (λ_{max} nm) 238; HRMS (ESI+) m/z 480.3443 [$(M+H)^+$; calculated mass for $C_{27}H_{41}N_7O^+$: 480.3445 amu].

4.2. Biological Screening

Inhibition assays of selected compounds against MRSA and MDRAB: Inhibition Assays were performed by overnight culturing the desired bacteria, MRSA or MDRAB, and subculturing at an OD_{600} of 0.01 into tryptic soy broth with a 0.5% glucose supplement (TSBG) for MRSA, or Luria-Bertani (LB) media for MDRAB. Stock solutions of a predetermined concentration of the selected compounds were prepared in the resulting bacterial suspension. Aliquots of 100 µL were distributed to the wells of a 96-well plate. Plates were covered and sealed with GLAD Press'n Seal, then incubated under stationary conditions at 37^{o}C for 24 hours. The media was then discarded, and the plates were washed thoroughly with water to remove any loosely adherent bacteria. Each well was then stained with 110 µL of crystal violet (0.1% solution), and allowed to sit for 30 minutes. The crystal violet was then discarded, and the wells were thoroughly washed with water again. The remaining stain was then dissolved in 95% ethanol (200 µL) and 125 µL was transferred to corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD_{540} value of each well. Blank wells were employed as background check.

Dispersion assays of selected compounds against MRSA: Dispersion assays were performed by taking overnight cultures of MRSA and subculturing at an OD_{600} of 0.1 into TSBG media. 100 µL aliquots of the resulting bacterial suspension were distributed to the wells of a 96 well plate. Plates were then covered and sealed with GLAD Press'n Seal followed by incubation under stationary conditions at 37^{o}C for 24h. The plates were unwrapped and the media was discarded, and the plates were washed thoroughly with water to remove any loosely adherent bacteria. Stock solutions of predetermined concentrations of the selected compounds were prepared in TSBG, and 100 µL was transferred from stock solutions into the 96-well plate. Media alone was added as a control. Plates were then rewrapped with GLAD Press'n Seal, and incubated under stationary conditions for 24 hours. The media was discarded from the plates and the wells were washed thoroughly with water. Each well was then stained with 110 µL of crystal violet (0.1% solution) at room temperature, and allowed to sit for 30 minutes. The crystal violet was then discarded and the wells were thoroughly washed with water again. The remaining stain was then dissolved in 95% ethanol (200 µL) and 125 µL was transferred to corresponding wells

of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD_{540} value of each well. Blank wells were employed as background check.

Broth microdilution method for antibiotic resensitization: Mueller-Hinton Broth (MHB) was inoculated ($5x10^5$ CFU mL^{-1}) with MRSA. Aliquots (5 mL) of the resulting bacterial suspension were distributed to culture tubes, and compound from a 100 mM stock solution was added to give the final desired concentration. Bacteria not treated with the tested 2-AIT served as a control. After sitting at room temperature for 30 minutes, 1 mL of each sample was transferred to a culture tube, and oxacillin sodium salt was added from a 128 mg mL^{-1} water stock solution to give a final concentration of 128 µg mL^{-1}. Rows 2-12 of a 96-well plate were filled (110 µL per well) from the remaining 4 mL bacterial subculture. After standing for 10 minutes, aliquots (200 µL) of the samples containing antibiotic were distributed to the corresponding first row wells of the microtiter plate. Row 1 was mixed 6-8 times, and then 100 µL was transferred from row 1 to row 2. This procedure was then repeated to dilute the rest of the wells, to which no antibiotic was added, to check for bacterial growth in the presence of compound alone. The plate was then covered and sealed with GLAD Press'n Seal, and incubated under stationary conditions at 37 ^oC. MIC values were then recorded as the lowest concentrations at which no bacterial growth was recorded, and a fold reduction was determined by comparison with control lane.

Checkerboard assay: MHB was inoculated with MRSA (5×10^5 CFU ml^{-1}) and 100 µL aliquots were distributed to all wells of a 96-well plate except for well 1a. Inoculated MHB (200 µL) containing a selected compound (at a concentration for 2x the highest concentration being tested) was added to well 1a, and 100 µL of the same sample was added to wells 2a-12a. Column A cells were mixed 6-8 times, and then 100 µL was withdrawn and transferred to column B. This process was repeated up to column G (column H was not mixed to determine the MIC of the antibiotic alone. Inoculated media (100 µL) containing antibiotic at 2x the highest concentration being tested was placed in wells A1-H1 and serially diluted, all the way until row 11 (row 12 was not mixed to determine the MIC of the compound alone). The plates were covered and sealed with GLAD Press'n Seal, and incubated under stationary conditions at 37^oC. After 16h the MIC values of both compound and antibiotic were recorded, as well as combination. The ΣFIC values were calculated as follows. $\Sigma FIC = FIC_{cmpd} + FIC_{antibiotic}$, where FIC_{cmpd} = [MIC_{cmpd} in combination]/[MIC_{cmpd} alone], and $FIC_{antibiotic}$ = [$MIC_{antibiotic}$ in combination]/[$MIC_{antibiotic}$ alone]. The combination is considered synergistic if $\Sigma FIC \leq 0.5$, indifferent if $0.5 < \Sigma FIC < 2$, and antagonistic if $\Sigma FIC > 2$.

5. *Acknowledgements*

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the position or the policy of the Government, and no official endorsement should be inferred.

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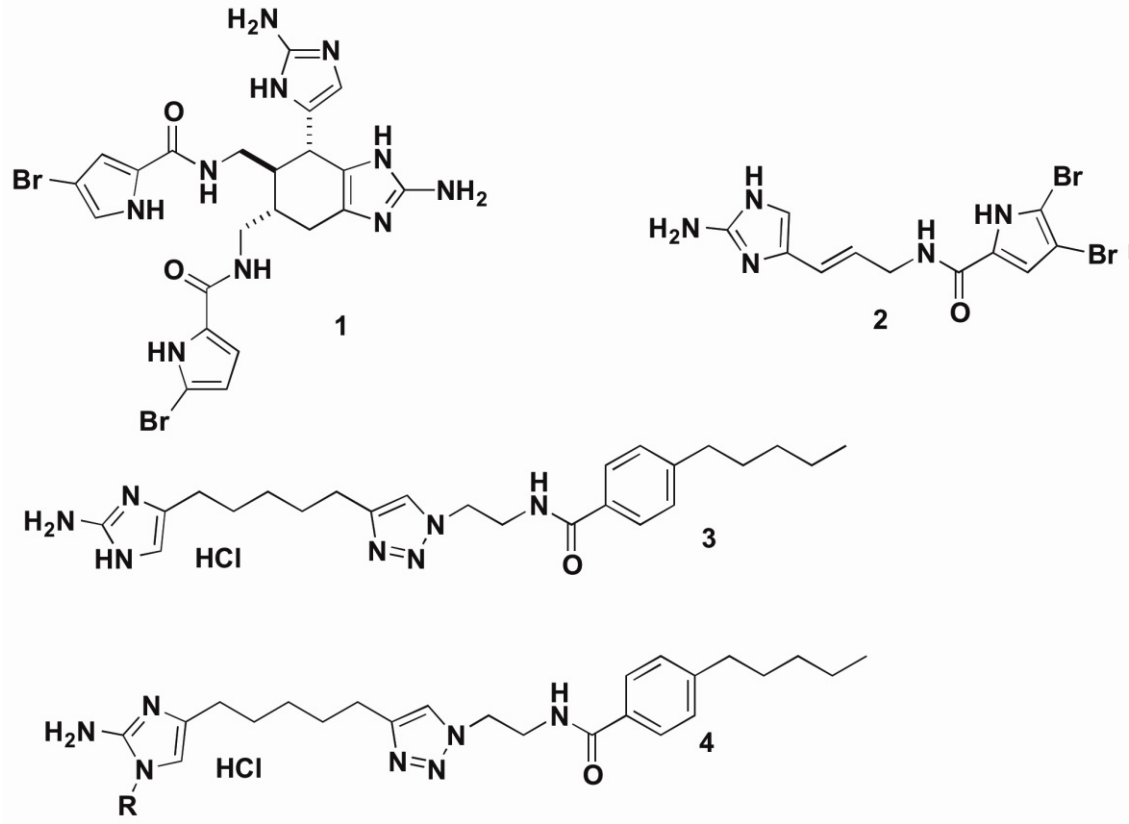


Figure 1

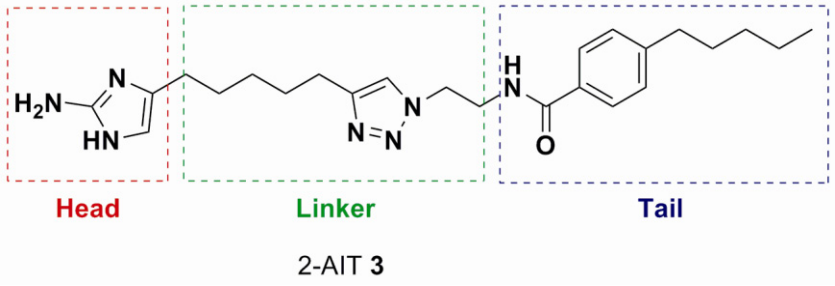
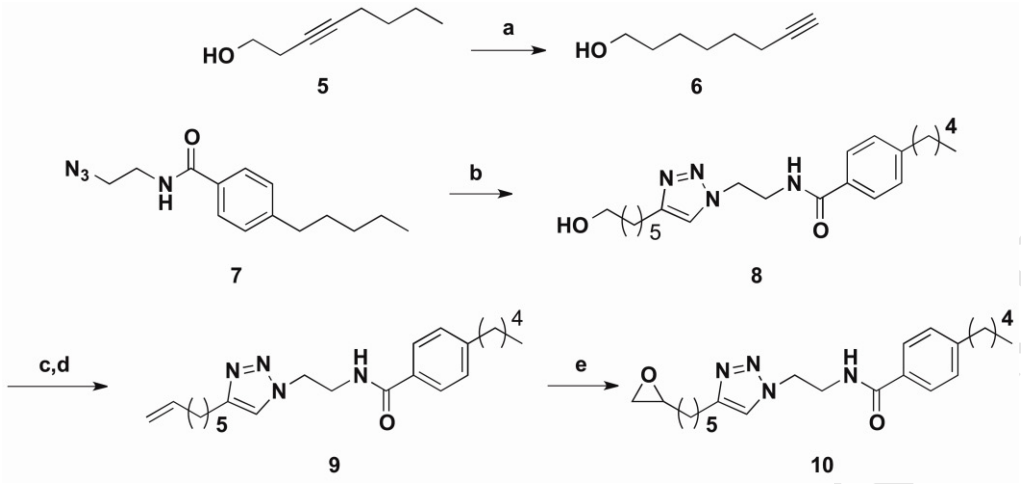
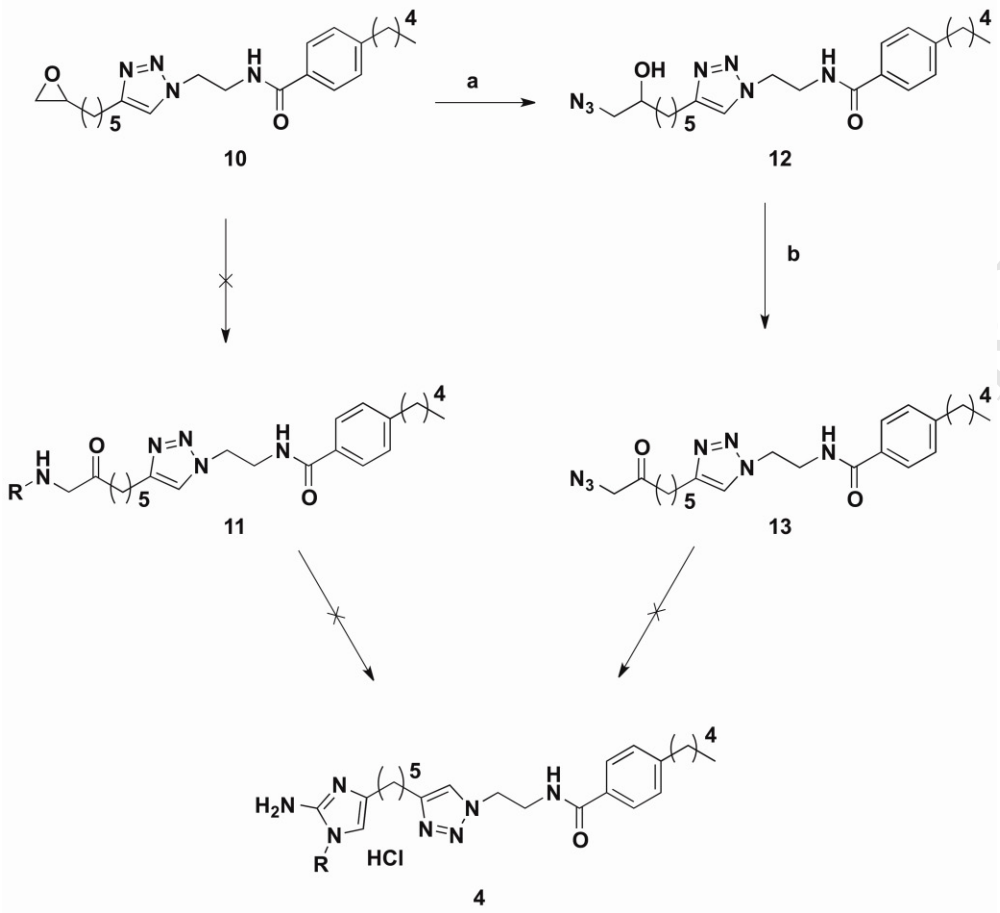


Figure 2

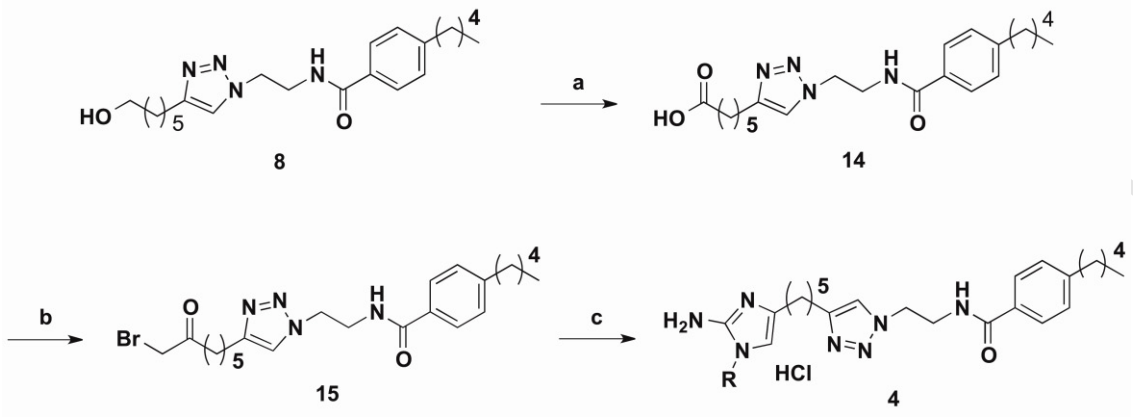
Figure 3



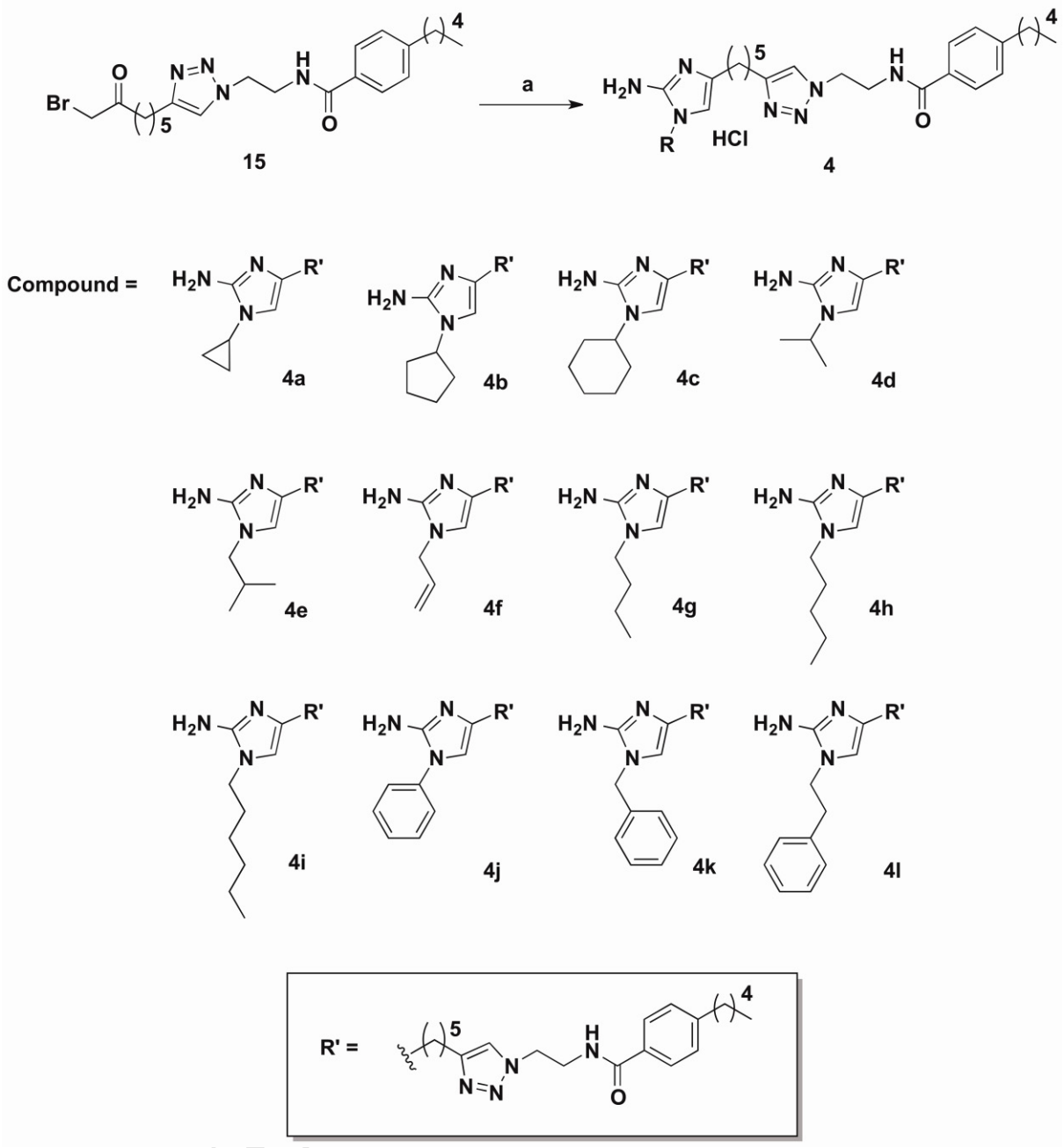
Scheme 1



Scheme 2



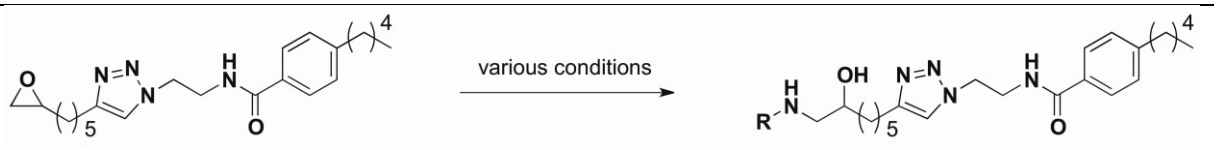
Scheme 3

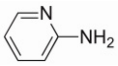
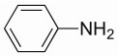
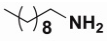
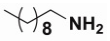
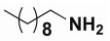
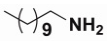
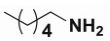
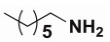
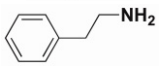


Scheme 4

Tables:

Table 1. Attempts at nucleophilic epoxide opening with primary amines.



Amine	Conditions	Equivalents	Yield
	a	1	NR
	b	1	NR
	b	1	NR
	c	8	NR
	d	8	55[a]
	e	8	35[a]
	f	8	NR
	d	8	NR
	e	8	NR

Conditions: a) toluene, reflux. b) Montmorillonite (K10), DCM, rt. c) DMF: H2O (1:1), rt. d) DMSO: H_2O (1:1), 60 ^oC. e) DMF, Montmorillonite K 10, 60 ^oC. e) DMF, 60 ^oC. f) DMF: DMSO/H_2O (4:1), Montmorillonite (K10), 60 ^oC.
[a] contained impurities after isolation

Table 2. Primary amines used for one pot 2-AI ring formation

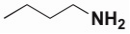
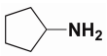
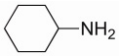
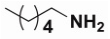
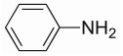
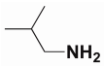
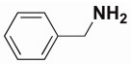
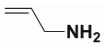
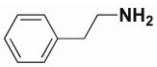
Amine	Product	Yield (%)	Amine	Product	Yield
	**4a**	16		**4g**	36
	**4b**	16		**4h**	26
	**4c**	28		**4i**	25
	**4d**	33		**4j**	26
	**4e**	14		**4k**	26
	**4f**	25		**4l**	10

Table 3. MICs and IC_{50}s of MRSA BAA-44 and MDRAB BAA 1605

Compound	MIC MRSA[a]	IC_{50} MRSA[a]	MIC MDRAB[a]	IC_{50} MDRAB[a]
4a	100	>100	>200	Toxic
4b	25	9.01 ± 2.39	100	33.7 ± 2.69
4c	25	18.71± 0.27	200	Toxic
4d	200	>100	>200	Toxic
4e	50	24 ± 3.88	200	98.59 ± 0.01
4f	50	13.2 ± 6.96	200	Toxic
4g	25	10.8 ± 3.18	100	53.8 ± 4.13
4h	12.5	4.14 ± 2.03	100	31.4 ± 4.13
4i	12.5	6.2 ± 0.84	100	26.2 ± 2.00
4j	25	25.1 ± 1.33	>200	49.8 ± 4.78
4k	12.5	7.16 ± 1.58	100	33.9 ± 2.76
4l	25	9.9 ± 4.54	100	39.2 ± 3.45

[a] values are in µM

Table 4. Biofilm inhibition and dispersion of lead compounds against MRSA BAA-44.

Compound	EC_{50}[a]
4b	34.47 ± 0.02
4h	45.06 ± 2.23
4i	40.96 ± 1.66
4k	39.61 ± 2.82
4l	32.96 ± 2.42

[a] values are in µM

Table 5. Resensization of MRSA to oxacillin

Compound	Resensitization	ΣFIC
4a	2 fold	0.501
4b	2 fold	0.562
4c	2 fold	0.625
4d	0-2 fold[a]	0.562
4e	4 fold	0.500
4f	2 fold	0.560
4g	0-2 fold[a]	0.530
4h	0 fold	0.530
4i	0 fold	1.000
4j	0-2 fold[a]	0.504
4k	0-2 fold[a]	0.515
4l	0-2 fold[a]	0.530

[a] 0-2 fold values obtained after experiments run in 4 replicate experiments

Caption Text:

Figure 1: 2-aminoimidazole containing compounds that display antibiofilm properties**.**

Figure 2: Head, linker and tail region of lead 2-AIT **3**

Figure 3: Retrosynthetic scheme towards synthesis of 1,4-disubstituted 2-aminoimidazole.

Scheme 1: Synthetic approach to intermidiate epoxide 10: *Reagents and condtions:* a) ethylenediamine, NaH, 86%. b) Compound **8**, $CuSO_4$, sodium ascorbate, t-BuOH/H_2O/DCM, 99%. c) i. DMSO, oxalyl chloride, DCM, -78^oC. ii. 6. iii. Et_3N. d) i. triphenylphosphonium bromide, KHMDS, toluene, -30 ^oC – rt. 66% over two steps. e) mCPBA, DCM, 0 ^oC – rt, 80%.

Scheme 2: Initial attempts at making compound 4: a) NaN_3, 2-methoxyethanol/H2O (8:1), reflux, 87%. b) DMSO, oxalyl chloride, DCM, -78 ^oC. ii. Intermediate alcohol. iii. Et_3N, 94%

Scheme 3: Synthesis of a 1,4-substituted 2-aminoimidazole/triazole via N-alkylation of an α-bromo ketone. a) Jones reagent, acetone, 0 ^oC, 70%. b) i. oxalyl chloride, DCM, cat DMF, 0 ^oC. ii. CH_2N_2. iii. HBr, 84% over 3 steps. c) i. R-NH_2, 95% EtOH, rt. ii. NH_2CN, pH adjusted to 4.3 using 0.1 N HCl_{aq} followed by reflux for 3 hours

Scheme 4: Synthesized 1, 4-2-AITs. a) i. R-NH_2, EtOH, 30 mins, ii. NH_2CN, pH adjusted to 4.3 using 0.1 N HClaq followed by reflux for 3 hours