ABSTRACT

CATHERINE SUZANNE REED. The Design and Synthesis of 2-Aminoimidazole Biofilm Modulators. (Under the direction of Christian Corey Melander.)

The following studies entail two classes of small molecules that modulate bacterial biofilm development. The first library of 2-aminoimidazole 1,4-disubstituted-triazole amide (2-AIT amide) analogues have been synthesized that exhibit the ability to tune activity to *E. coli*. Some of the analogues in the library promote the growth of *E. coli* biofilms, while there are two leads, one that both inhibits and disperses *E. coli* and the other that can only inhibit *E. coli* biofilm formation. All the analogues of the 2-AIT amide library modulate *E. coli* biofilms without killing planktonic bacteria. The second class of small molecules were synthesized based on a 2-aminobenzimidazole “parent” library that were not able to modulate either Gram-negative or Gram-positive bacteria, but did exhibit antibiotic activity towards both. By removing the aniline amide moiety from the lead compound of the “parent” library, the new 2-AI small molecules maintained antibiotic activity in addition to being able to disperse Gram-negative bacteria. The new class of 2-AI small molecules can be considered antibiotics that disperse, which is highly important due to the increased resistance (upwards of 1000 times greater) that biofilms have to modern day antibiotics as compared to their planktonic state. Given the profound impact biofilms encompass, it is important to discover small molecules that have the ability to modulate bacterial biofilms.
The Design and Synthesis of 2-Aminoimidazole Biofilm Modulators

by
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BIOGRAPHY

Catherine Suzanne Reed was born in Gaithersburg, Maryland to the parents of Frederick A. Reed III and Carolyn S. Chimera-Reed. Four and a half years later there was an addition to the family, a little brother Patrick A. Reed. The following summer her family moved from Montgomery Village, Maryland to Frederick, Maryland where they still reside today. She participated in many sports growing up, ranging from field hockey to gymnastics. She graduated from Urbana High School in June 2003 and then moved her life to North Carolina to attend the University of North Carolina Wilmington (UNCW). She was very involved in extra-curricular activities in college and was a student ambassador at UNCW. She graduated cum laude from UNCW in May 2007 with a BS in chemistry and a minor in mathematics. In August of 2007 she moved from Wilmington, NC to Raleigh, NC to attend graduate school at North Carolina State University in chemistry. She joined Dr. Christian Melanders research group in November of 2007.
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CHAPTER 1

INTRODUCTION

1.1 Bacterial Biofilms

In the past 20-30 years, there has been a realization that bacterial biofilms account for a significant number of bacterial infections.\[^1\] A biofilm is a community of bacteria living in an organized community attached at a solid-liquid interface encased in a protective extracellular matrix.\[^2,3\] It is estimated by the U.S. National Institutes of Health that over 80% of microbial infections that occur in the human body are mediated by the formation and persistence of biofilms.\[^4\] Biofilms in nature are rarely composed of a single bacterial species; instead several species are often present in the biofilm community. Common infections that are associated with biofilms include lung infections in patients who suffer from cystic fibrosis (CF), tooth decay, burn wounds, and otitis media (the common ear infection) (Table 1.1).\[^4\]

**Table 1.1**: Common biofilm-forming bacteria and their respective diseases.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram +/-</th>
<th>Biofilm associated disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>-</td>
<td>Burn wound, trauma infection</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>Urinary, catheter infection</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>Cystic fibrosis lung infection</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>-</td>
<td>Cystic fibrosis lung infection</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>-</td>
<td>Gastrointestinal infection</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>-</td>
<td>Respiratory infection</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>Burn wound, catheter, trauma infection</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>+</td>
<td>Sepsis, catheter infections</td>
</tr>
<tr>
<td><em>Streptococcus mutants</em></td>
<td>+</td>
<td>Tooth decay</td>
</tr>
</tbody>
</table>

In the past 20 years, biofilms have become recognized as a serious threat to society and the medical community. Bacterial biofilms are responsible for about 65% of nosocomial
infections resulting in treatment costs of > $1 billion annually.[5] Biofilms develop preferentially on inert surfaces, and commonly occur on indwelling medical devices (IMDs) such as urinary catheters, heart stints, and joint replacements.[6] Biofilms on IMDs are a continual source of infection, which necessitates the complete removal of the device from the patient. Removal of the device increases both the trauma and cost to the patient.[5] Modern day acute infection can often be treated effectively with antibiotics, however standard antimicrobial treatments typically fail to eradicate biofilms resulting in chronic infection. Biofilm mediated infections are known to be upwards of 1000 times more resistant to antibiotics than its planktonic (free floating) state.[7] There are currently few chemical compounds that have specific anti-biofilm properties, which presses for the urgency in the development and discovery of new agents to effectively eradicate biofilms.[4]

1.2 Biofilm-Forming Bacteria

1.2.1 Acinetobacter baumannii

The genus Acinetobacter is an important group of Gram-negative bacteria that is associated with a wide range of infections. Since the 1980s, 17 named species have been recognized with Acinetobacter baumannii as the most common species involved in hospital infections.[8] Some of these hospital infections include ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, urinary-tract infections, and blood stream infections. A. baumannii based infections are difficult to treat because of high resistance to antibiotics. Many studies have discovered the occurrence of multidrug resistant (MDR) strains of A. baumannii in hospital environments.[9]
Since 2001, many American service members have been deployed to Afghanistan and Iraq. The living conditions and stresses brought on by war result in epidemics of infection that involve both civilian populations and soldiers. Despite the efforts of developing better sanitation and hygiene practices, infectious diseases remain an important concern in war-torn countries. Many soldiers have been wounded in action with these wounds then becoming contaminated with bacteria. The bacteria found in the wound infections are often multidrug resistant and include the species *A. baumannii*. Since 2003, the occurrence of multidrug resistant *A. baumannii* has increased significantly, with a majority of the cultures being from war wounds. Transmission of *A. baumannii* occurs throughout the military health care system as well.

1.2.2 *Escherichia coli*

*Escherichia coli* is an extremely diverse gram-negative bacteria that has the ability to colonize various habitats. For example, *E. coli* has the ability to form a beneficial symbiotic relationship with their host providing key nutrients and signals, while on the other hand can diverge, taking on a pathogenic nature with the ability to cause disease to the host. These strains of *E. coli* have been broadly characterized as either diarrheagenic or extraintestinal *E. coli* (ExPEC). Diarrheagenic *E. coli* has the ability to cause gastrointestinal disease, but is not known for causing disease outside of the intestine. On the other hand, ExPEC has been known to colonize other places such as the blood, central nervous system, and urinary tract, all resulting in disease.

Uropathogenic *E. coli* is the most common strain of ExPEC associated with human disease. It is the primary cause of both community-acquired urinary tract infections (UTI)
and of Nosocomial UTIs (70-95%).[12] UTIs result in $1.6$ billion in medical expenses in the United States each year, especially due to the recurrence of UTIs.[13] Once the bacteria are inside the urinary tract, it can colonize the bladder causing cystitis, and can also ascend through the ureter to the kidneys causing pyelonephritis. These infections reside in normal sterile environments that usually have host responses to infections including generation of antimicrobial compounds, but the bacteria have evolved a number of strategies to evade the immune responses that enables effective colonization and persistence of infection.[13]

There are many other diseases that are associated with *E. coli* infections. *E. coli* usually is not regarded as a common cause of pneumonia, however it plays a major role in nosocomial pneumonia (60-70%). It is usually the third or fourth commonly isolated Gram-negative bacteria in nosocomial pneumonia.[12] There are approximately 34 million surgeries performed annually and anywhere between 300,000 to 800,000 of those sites becoming infected. *E. coli* is the 4th most common bacteria to cause the surgical site infections, which can lead to longer hospital recoveries. It is also the leading cause of neonatal meningitis, and can cause both abdominal and pelvic infections.[12]

### 1.2.3 Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacteria responsible for many difficult to treat infections. It is especially troublesome in nosocomial infections and is the leading cause of nosocomial bacteremia.[14] Other nosocomial related problems due to MRSA include skin infections, surgical site infections, catheter infections, IMD infections, and pneumonia. Current procedures for infection control in hospital are not successful, resulting in an increase of MRSA related diseases. For example, in the 1980s 1-
5% of S. aureus isolates were methicillin-resistant, and today 60-80% of S. aureus strains isolated from hospitals are methicillin-resistant.\textsuperscript{[15]} Over the years MRSA has increased, showing up in community-acquired infections, on children from day-care facilities, contact sport athletes, in prisons, and intravenous-drug users. Currently, 50% of S. aureus infections outside of the hospitals are now methicillin-resistant.\textsuperscript{[15]}

1.3 Biofilm Growth Model

Bacteria strains, both gram negative and gram positive, require certain environmental conditions to establish a biofilm. Many studies have been performed to determine the way in which these diverse bacteria establish biofilms.\textsuperscript{[5,7]} While each bacteria may only grow a biofilm under certain conditions, there are also many similarities between all biofilm producing bacteria. Therefore, it can be stated that development of a biofilm occurs in a five-stage process (Figure 1.1). Reversible attachment of planktonic bacteria to a surface suitable for growth is the first step in biofilm growth. Since these bacteria cells are not committed to the process of biofilm growth, they sometimes may leave the surface in their planktonic free-floating form.
Figure 1.1: Biofilm growth model.

During this stage the bacteria also exhibit many species-specific behavior on the surface, such as rolling, creeping, and aggregate formation.\cite{16} Since the bacteria exists in its planktonic form, antibiotics and antimicrobials can kill the bacteria before a biofilm is formed. It is also proposed to be the stage in biofilm development where anti-biofilm modulators elicit their effects by preventing attachment of the bacteria to the surface, thus keeping the bacteria in its planktonic state. As the bacteria mature to stage two, the attachment to the surface goes from reversible to “irreversible” due to the secretion of an exopolymeric substance (EPS).\cite{17} The EPS is composed of a variety of biomolecules from both the bacteria and the surrounding environment and provides space for the biofilm to mature. The production of the EPS is considered one of the most distinguishing characteristics of a biofilm because it is suggested that the EPS prevents the access of some antibiotics and microbicides to the bacterial cells within the biofilm.\cite{5} This attachment
initiates expression of biofilm-specific genes, leading to a ‘biofilm phenotype’, which distinguishes the biofilm bacteria from their free-floating planktonic counterparts.[6] Stage three shows early maturation of the biofilm, which is observed as the transformation into three-dimensional architecture. Development continues to what is considered the fully mature biofilm. The topography of the biofilm becomes distinct with complex architecture such as pillars, cavities, and hollow channels. These features are necessary for nutrient adsorption, waste disposal, and transport systems to funnel water and bacteria through the biofilm.[1] The last stage of the biofilm developmental model is detachment of the bacteria from the biofilm. The bacteria, which have been documented to be similar to their planktonic counter-parts, is dispersed to colonize on new surfaces and repeat the cycle.[17]

1.4 Mechanisms of Antibiotic Resistance in Biofilms

The medical community faces a great problem due to the known antibiotic resistance of biofilm-driven infections. Great efforts have been directed towards the study of the effects of antimicrobial agents on biofilms, and many mechanisms have been suggested. It has been postulated that the EPS matrix of a biofilm acts as a barricade to antibiotics to the bacterial cells in the community.[18] To probe this hypothesis studies have been performed that monitor antibiotic concentration at the base of the biofilms. One study used the antibiotic ciprofloxacin against Pseudomonas aeruginosa biofilms and monitored the concentration of the antibiotic with infrared spectroscopy.[4] The results from this study concluded that the biofilm was able to significantly reduce penetration of the antibiotic to the surface. This observation is not consistent across all antibiotics and penetration rates depend on the antibiotic used and are not correlated to antibiotic susceptibility.[6] A study
utilizing a fluorescent analogue of tetracycline concluded that a strain of *Escherichia coli* biofilm shows tetracycline mediated fluorescence throughout the entire biofilm after 7.5-10 minutes of exposure to the antibiotic via use of confocal laser scanning microscopy (CLSM).[19] However, the biofilms were less susceptible to the antibiotic than were their planktonic counter-parts even with complete penetration of the antibiotic into the biofilm.[19] Limited penetration of some antibiotics into biofilms is believed to be due to the EPS matrix, which is a complex structure comprised of polysaccharides and occasionally small peptides and DNA.[4] Thus, the EPS could behave as an adsorbent for charged compounds or biocides, which would limit the penetration of these molecules into some bacterial biofilms. These studies suggest that the EPS can act as a barrier to chemicals, allowing for the survival of biofilm bacteria in presence of antibiotics that would eliminate their planktonic form.

A second resistance mechanism of biofilms is the various microenvironments within the biofilm that creates many layers of defense mechanisms. When bacteria undergoes starvation of certain nutrients it slows its growth, and transition from exponential to slow growth is correlated to an increase in resistance to antibiotics.[6] Recent studies have been able to examine the effects of growth rate to survival of a biofilm cell against antibiotics. For example, after observing growth rates of planktonic vs. biofilm cells of bacteria (*P. aeruginosa, E. coli, S. epidermidis*) it was concluded that a slower growth rate of biofilm cells protects the cells from antibiotic agents.[20-22] The bacteria cells growing in biofilms experience nutrient limitations, which suggests that the physiological change can account for the increased resistance to antibiotics.[20,21] However, as growth rate is increased, the
planktonic cells become more susceptible to the antibiotic than the biofilm cells, supporting that a mechanism other than growth rate provides resistance to antibiotics for biofilm cells.\textsuperscript{[5]} Slow growth of cells has also been attributed to a general stress response to various environmental stresses, such as heat shock, and changes in pH.\textsuperscript{[5]} Hypermutation, which is when bacteria mutates at a higher rate to survive under stress, is an effect seen in biofilms more frequently than planktonic populations. This effect also elicits resistance to antibiotics due to development of specialized efflux pumps in the biofilm.\textsuperscript{[23]} The complex structure of biofilms, water channels for the transport of nutrients and waste products in and out of the biofilm, creates different environments within the biofilm. For example, due to the acidic nature of some of the cellular waste, there are localized areas of low pH within the biofilm. The acidic conditions are suggested to aid in the survival of biofilms by reducing the activity of antimicrobials.\textsuperscript{[22]}

Bacteria populations produce persister cells, which neither grow nor die in the presence of antimicrobial compounds.\textsuperscript{[24]} Currently, the nature of persister cells is unknown, however persisters are not mutants and do not represent a special stage in the cell cycle.\textsuperscript{[18]} It is believed that the existence of persister cells attribute to the reoccurrence of biofilm-associated infections. A majority of the cells in a biofilm are not necessarily more resistant to antimicrobial treatment than planktonic cells, but persister cells survive this treatment. If the concentration of the antibiotic drops, or therapy is discontinued, the persister cells which are embedded within the biofilm matrix and cleared by the immune system are able to repopulate the biofilm and re-infest the host, which explains the reoccurrence of biofilm-associated infections.\textsuperscript{[24]}
1.5 Cell–to–cell Communication in Bacteria

Bacteria communicate with one another using chemical signals that are synthesized and secreted by the bacteria. These signals, known as autoinducers, increase in concentration as a function of increasing cell-population density. This phenomenon, known as quorum sensing (QS), allows the bacteria to monitor the environment for other bacteria and to alter gene expression in an attempt to ensure bacterial survival. Bioluminescence, biofilm formation, virulence factor expression, and antibiotic expression are some of processes that are controlled by quorum sensing in bacteria.

1.5.1 Quorum Sensing in Gram-negative Bacteria

The first quorum-sensing system was discovered in the gram-negative marine bacterium Vibrio fischeri, as being involved in the control of bioluminescence in the light organ of the Hawaiian squid Euprymna scolopes. The quorum-sensing system in V. fischeri is considered the prototype of most systems present in Gram-negative bacteria. Two regulatory proteins, LuxI and LuxR, control the production of light, which the squid uses to avoid predators. LuxI is the enzyme that synthesizes the acyl-homoserine lactone (AHL) autoinducer that freely diffuses in and out of the cell. The production of the AHL increases as the cell density of LuxI increases, resulting in a high concentration of AHL both inside and outside of the cell. When the AHL signal concentration reaches a certain threshold, it binds to the LuxR protein and activates transcription of the operon encoding luciferase (required for light production). The luxI gene is upregulated by the complexation of
LuxR-AHL, which leads to further production of LuxI flooding the environment with AHL, thus leading to a rapid increase in light (positive feedback loop) as shown in Figure 1.2.\textsuperscript{[26]}

![Diagram of Gram-negative QS Pathway](image)

**Figure 1.2:** Gram-negative QS Pathway.

AHL autoinducers all share the same homoserine lactone core and differ by distinct acyl side chains (Figure 1.3 A). These autoinducers are produced from S-adenosyl methionine (SAM) and specific fatty acyl carrier proteins by LuxI-type AHL synthase. Structural studies indicate each enzyme possesses an acyl-binding pocket that fits a particular acyl side-chain.\textsuperscript{[28]} The structures of the LuxR proteins also suggest that the proteins possess specific acyl-binding pockets that allow for binding and activation only by its equivalent AHL signal. AHLs encode a diverse range of functions including extracellular protease production, cell division, motility, and biofilm differentiation.\textsuperscript{[25]}
1.5.2 Quorum Sensing in Gram-positive Bacteria

Although there are many parallels that exist between Gram-negative and Gram-positive quorum sensing systems, there are important differences that exist. The differences between quorum sensing systems in gram-positive bacteria are in the structure of their autoinducer molecules and in the mechanism of signal recognition and sensing.\textsuperscript{[25]} Gram-positive bacteria use peptides to communicate (autoinducing peptide, AIP) in comparison to gram-negative AHL signals. These oligopeptide autoinducers (Figure 1.3 B) range from 5 to 17 amino acids and are often modified through the incorporation of lactone and thiolactone rings, lanthionines, and isoprenyl groups.\textsuperscript{[28]} AIPs are detected by a two-component signal transduction system, in which the AIP binds to a membrane-bound histidine kinase sensor and the binding information is relayed through the cell via a phosphorylation cascade. However, AIPs do not freely diffuse in and out of the cell, instead oligopeptide exporters mediate the release of AIP signals. Just like AHLs, AIPs have a core peptide and contain...
subtle variations through different gram-positive bacteria. Specificity is achieved through
binding to their related receptor.\textsuperscript{[29]} The importance of studying Gram-positive quorum
sensing systems has increased due to the virulence nature of gram-positive organisms. The
two-component system in \textit{Staphylococcus aureus} is a well-studied example of Gram-positive
quorum sensing which uses a biphasic strategy to cause disease and is regulated by the Agr
quorum sensing system.\textsuperscript{[26]}

\section*{1.5.3 Quorum Sensing Circuits and Cross-Talk}

It is apparent that there is high specificity between a specific autoinducer signal and
its receptor, but several bacteria produce more than one signal and possess multiple quorum-
sensing circuits. The first observation of multiple quorum-sensing systems was in the Gram-
negative marine bacterium \textit{Vibrio harveyi}.\textsuperscript{[26]} The system consists of three autoinducers that
work in parallel to channel the information into a shared regulatory pathway. This
arrangement can be beneficial because it provides a way for the cells to synchronize their
responses to different signal. Multiple quorum sensing systems are present in other bacterial
species, such as \textit{P. aeruginosa} and \textit{S. aureus}. The quorum-sensing circuits in \textit{P. aeruginosa}
are arranged in series and ensures that only certain genes are expressed at a given time of a \textit{P. aeruginosa} infection.\textsuperscript{[30]}
Bacteria are known to exist in multispecies communities, and thus communication between the different species is required for the community to sustain itself. The only signal known to function as interspecies cell-cell communication, is autoinducer-2 (AI-2), a small molecule synthesized by the luxS gene.\(^{[26]}\) The luxS gene produces 4,5-dihydroxy-2,3-pentanedione (DPD), which undergoes a variety of rearrangements to synthesize the AI-2 (Figure 1.4), and different species of bacteria recognize their distinct rearrangements of DPD. The luxS gene has been identified in more than 55 species of Gram-negative and Gram-positive bacteria.\(^{[25]}\) For example, in the oral cavity, the organization and interactions between different bacteria is mediated by AI-2 signaling.\(^{[25]}\) It also appears that AI-2 is involved in intraspecies signaling and regulations of phenotypes. The control of motility in bacteria, which can change the biofilm architecture, has been demonstrated in several species including *E. coli* K-12 and is a result of the AI-2 signal. AI-2 has also been shown to regulate the expression of virulence genes that are involved in some *E. coli* infections.\(^{[25]}\)
1.6 Quorum Sensing Inhibition

Quorum sensing, which plays a major role in biofilm-mediated infections, is thought to be among some of the reasons that antimicrobial therapies are ineffective in eradicating the infections. There have been great efforts in finding ways to block these quorum-sensing systems as a way to mitigate the biofilm formation and thus eliminate the infectious bacteria. Genetic knockout experiments have provided proof of concept that agitating the quorum-sensing cascade can affect biofilm formation, and thus disruption of the system with a small molecule is needed to move these strategies into clinical trials. The quorum-sensing cascade reveals many possible targets for disruption by small molecules, such as blocking production, binding of the QS signal, or blocking the downstream biochemical pathways resulting from signal binding. The quorum-sensing cascade begins with the production of AHL. For example there are bacterial enzymes that are known to degrade AHL signals, which results in decreased concentration of AHLs. Under this scenario Gram-negative bacteria would be unable to express their pathogenic phenotypes because their AHL concentration would not be able to reach the concentration threshold necessary to induce gene expression. Enzymes are not common antimicrobial agents in the clinic, but they may be applicable in the fight against Gram-negative quorum-sensing pathogens.
Figure 1.5: Gram-negative QS Inhibitors.

A second potential to disrupt the quorum-sensing cascade is to disrupt the binding of the AHL signal to its cognate receptor, generally a LuxR homologue. This possibility occurs in nature, because many organisms are in direct competition with bacteria (Gram-negative) and produce analogs of AHLs to disrupt the quorum-sensing cascade to avoid disease.\(^4\) One example of this is the marine macroalga, *Delisea pulchra*, that produces a set of halogenated furanones (Figure 1.5 A) that inhibit the quorum-sensing circuit of bacteria that can colonize and cause disease on the organism. Derivatives of the halogenated furanones have been synthesized (Figure 1.5 B) and shown to inhibit *P.aeruginosa* biofilm-sensing circuits and
thus affect biofilm structure.\textsuperscript{32} Some of the synthetic derivatives have caused promotion of the quorum-sensing cascades rather than inhibition. The Blackwell group utilized microwave chemistry to develop many AHL derivatives and one was found to be a potent inhibitor (Figure 1.5 C) of the \textit{P. aeruginosa} quorum-sensing circuit.\textsuperscript{33,34} Model studies of some of the AHL receptor inhibitors suggest that they could function by preventing conformational change of the LuxR into its active DNA binding conformation, which prevents gene expression.\textsuperscript{4} On the other hand, there are a few problems associated with the development of AHLs as small-molecule controls of biofilms. One problem is the chemical reactivity of the lactone moiety since they are prone to hydrolysis. Groups have focused their research towards the modification of the lactone moiety in hopes of overcoming the lack of stability the lactones possess in physiological solution.\textsuperscript{35-37} For example, Spring and co-workers synthesized derivatives that featured 2-substituted cyclopentanone and cyclohexanone (Figure 1.5 D) in place of the lactone, and found that these compounds did inhibit biofilm formation.\textsuperscript{38}

The development of peptides as quorum-sensing inhibitors in Gram-positive bacteria has recently been studied. The RNAIII-inhibiting peptide (RIP) has been shown to be widely effective as a quorum-sensing inhibitor against \textit{S. aureus} in both \textit{in vitro} and \textit{in vivo} models.\textsuperscript{39} The mechanism of inhibition for RIP is that it competes with RNAIII-activating protein (RAP) for binding to the protein “target of RAP (TRAP)” which prevents phosphorylation of TRAP, thus inhibiting the quorum-sensing circuit in \textit{S. aureus}. The RIP peptide has shown \textit{in vivo} activity in numerous animal models including the ability alleviate infections caused by multi-drug resistant bacterial strains.\textsuperscript{40} The peptide has even been used
to coat surfaces of medically implanted devices to prevent biofilm formation. Thus far, RIP shows no signs of toxicological side effects and no reports of resistance have been reported.\textsuperscript{[1]} However RIP is not optimal as a drug candidate.\textsuperscript{[4]}

There are many drawbacks to targeting quorum-sensing cascades for biofilm inhibition. First of all there are many differences between the quorum-sensing pathways of gram-negative and gram-positive bacteria. The same compounds that inhibit gram-negative quorum sensing would not be effective against gram-positive quorum sensing pathways due to the differences between them, and vice-versa. Thus, targeting quorum-sensing inhibition is not ideal because the compounds are not able to inhibit over both order and phylum. Also, these compounds have not displayed the ability to disperse preformed biofilms, which is an important factor in the discovery of anti-biofilm modulators.

1.7 Chemical Library Screening

Many groups have utilized the process of high-throughput screening (HTS) to identify a number of biologically active molecules.\textsuperscript{[4]} HTS is advantageous because it rapidly screens thousands of diverse compounds in order to identify hits in the assay. The Wood group was in search of natural products that were able to inhibit biofilm formation. The group screened a total of 13,000 compounds from 176 different plant families for biofilm inhibition.\textsuperscript{[41]} From this library of 13,000 compounds, ursolic acid \textbf{1.1 (Figure 1.6)}, from \textit{Diospyros dendo} was found to inhibit biofilm formation \textit{E. coli} without affecting its growth rate. Ursolic acid is a nontoxic ingredient of many medicinal plants and has a broad range of pharmacology effects. The Wood group discovered that 10 (\textmu g/mL) of ursolic acid inhibited \textit{E. coli} biofilm formation by 20 fold and ursolic acid was not toxic to \textit{E.coli, P. aeruginosa, V. harveyi}.\textsuperscript{[41]}

Following this discovery, the Wood group performed DNA microarrays to identify the mechanism of the inhibition on a genetic basis. They found that ursolic acid also affected the regulation of genes associated with biofilm formation.

![Chemical structures](image)

**Figure 1.6**: Lead antibiofilms compounds from chemical library screening.

The Hergenrother group screened over 4,500 compounds for inhibition activity against *P. aeruginosa* biofilms. Each compound was screened at 50µM and they considered the compound a “hit” if it reduced the growth of the biofilm by at least 30%. The preliminary screen identified 6 compounds that reduced biofilm production and after further studies, ferric ammonium citrate, 1.2 (FAC) (Figure 1.6) was found to both inhibit and disperse biofilms. Ammonium chloride, citric acid, ammonium citrate, and sodium citrate were tested to see if either the ammonium or the citrate were responsible for the inhibition of the *P. aeruginosa* biofilms. Neither the ammonium nor the citrate was responsible for the inhibition, however the iron was found to be the source of biofilm inhibition. Since simple iron salts inhibited biofilms, the Hergenrother group was interested in discovering if chelated iron sources showed the same results. They screened commercially available iron chelators loaded with iron so that neither the free chelator nor iron would be present in
solution. Any activity would be a result of the *P. aeruginosa* to mobilize the iron from the chelated form. Two iron chelators showed similar results as the ferric ammonium citrate in their ability to inhibit biofilm formation. The two leads were also assayed as their free states, and did not affect biofilm formation at concentrations up to 100µM, and at concentrations above this slight bacterial growth inhibition was found. This experiment confirmed that the iron, and not the chelator, was responsible for the biofilm inhibition.

The high throughput screen of over 66,000 compounds by Clardy and Junker was performed to identify compounds that prevent biofilm formation of *P. aeruginosa* without affecting planktonic bacteria growth.\[44\] They utilized a luminescence-based biofilm screen rather than a crystal violet screen to identify the compounds that disrupted biofilm formation. The 66,000 compounds represented many different scaffolds and libraries, and after preliminary screens 193 compounds made it through to further screening. In the end, the luminescence high throughput screen identified a total of 30 compounds that fell into six structural classes as biofilm inhibitors with IC$_{50}$ values lower than 20µM with the lead compound (*Figure 1.6, 1.3*) exhibiting an IC$_{50}$ value in the nanomolar range. These 30 compounds were selective for biofilm inhibition and had no affect on bacteria growth.\[44\]

### 1.8 Natural Products

Biofilms have the ability to grow on many different surfaces that can lead to problems in many different areas, such as the food, naval, dental, medical, and water treatment industries. Recent studies have lead to the discovery of antibiofilm modulators from natural products. Cranberry juice and cranberries have been shown to reduce urinary tract infections of *E. coli* and inhibit the formation of oral streptococci.\[45,46\] Other studies have shown that
glass plates covered with salicylic acid polymers inhibit biofilm formation.\textsuperscript{[47]} Garlic extracts have demonstrated the ability to inhibit quorum-sensing gene expression in \textit{P. aeruginosa}. Recent studies have shown that extracts of garlic make \textit{P. aeruginosa} biofilms susceptible to tobramycin and phagocytosis by polymorphonuclear leukocytes, resulting in an improved outcome in respiratory infections.\textsuperscript{[48]} These natural products all show various degrees of antibiofilm properties against numerous strains of bacteria.

Much attention has been directed towards marine natural products as chemical controls of biofilms. Sponges of the \textit{Agelas} genus are important components of the Caribbean coral reef because of their abundance in a variety of habitats.\textsuperscript{[49]} These sponges appear to protect themselves from predators via chemical defenses because their structural defenses were ineffective feeding deterents.\textsuperscript{[50]} The relatively clean surfaces that are observed can be explained by the sponge’s ability to inhibit attachment of non-commensal bacteria and biofilm formation, preventing both infection and biofouling.\textsuperscript{[51]} Biofouling is known as the accumulation of microorganism, algae, or plants on a surface, and has been known to have a significant economical impact on the shipping industry because it can reduce the performance leading to an increase in fuel.\textsuperscript{[52,53]} This process also has the ability to occur on aquatic organisms, such as the sea sponge. Inhibition of biofouling is beneficial to the sponge due to the negative consequences such as feeding obstruction, tissue degradation, and increased change in dislodgement and mortality.\textsuperscript{[54]} Studies have shown that the metabolites of the sponge act as defense against predators, and secondary metabolites often posses antimicrobial properties.
The *Agelas* genus is distinct because they contain primarily brominated pyrrole alkaloids that are structurally unique as secondary metabolites.

![Chemical structures](image)

**Figure 1.7:** Marine alkaloids isolated from *Agelas conifera*.

Brominated alkaloids (Figure 1.7) such as sceptrin (1.4), oroidin (1.5), and ageliferin (1.6) have been isolated from *Agelas conifera*.\(^{[50]}\) Ageliferin was first isolated in 1986 by Rinehart and has been the subject of numerous synthetic efforts due to its antiviral activity.\(^{[55]}\) The secondary metabolites of *Agelas conifera* have garnered much attention because of their ability to inhibit biofilm formation.\(^{[56]}\) This is important because it shows that the secondary metabolites are able to inhibit biofilm formation of both gram-negative and gram-positive bacteria. Although ageliferin, bromoageliferin (1.7), and dibromoageliferin (1.8) possess great antimicrobial properties, they are not an ideal target for medicinal purposes because of the
difficulty and length of the synthesis. Instead, investigation of the structure-activity relationship (SAR) of the three-ageliferin alkaloids is more ideal for identification of new antibiofilm modulators.

REFERENCES


2.1 Introduction

Given the central role biofilms play in infectious disease, the Melander group has initiated a research program toward the discovery and development of compounds that can both inhibit and disperse bacterial biofilms.

Figure 2.1: Synthetic analogues derived from bromoageliferin.
Marine natural products isolated from the *Agelas* genus have shown extraordinary biological activity and possess stunning molecular architecture.[1] More specifically, bromoageliferin, isolated from *Agelas conifera* has been reported to possess anti-biofilm activity against *R. salexigens* and is a member of the oroidin class of natural products. These biologically active compounds are characterized by a 2-aminoimidazole (2-AI) subunit and have been the structural inspiration for our work.

First, our group synthesized two initial compounds to test the importance of the 2-AI head group and the bicyclic core of bromoageliferin. These compounds, trans-bromoageliferin analogue (TAGE) and cis-bromoageliferin analogue (CAGE) (Figure 2.1) were tested against two strains of *P. aeruginosa* (PAO1 and PA14), which is a γ-proteobacteria that is in the same phylum as *R. salexigens*, and was shown to inhibit both strains.[2] Next, our group focused on the synthesis of compounds based on the natural product oroidin, and SAR studies were performed to identify the structural features that were essential for biological activity. The synthesis of a 50-compound library based on oroidin concluded in the identification of a new lead compound, dihydrosventrin (DHS) as an inhibitor of *P. aeruginosa* biofilms.[3] The synthesis of a new library was based off the lead compound DHS by exchanging the pyrrole subunit with a triazole subunit. This new 2-AI/triazole library led to the discovery of compounds that were able to both inhibit and disperse bacterial biofilms across order, class, and phylum, with the most active compound being SPAR (Figure 2.1).[4]
Since we have already identified a number of 2-AI’s that can be designed to inhibit and disperse biofilms of both Gram-negative and Gram-positive strains,\textsuperscript{[5-7]} we attempted to target activity to a specific bacterial population. Recent results have shown that bioactivity can be tuned to a certain strain by modulation of the functional groups of a 2-AI. With this in mind, we wanted to find a way to rapidly generate diversity in the 2-AI scaffold. This was accomplished through utilization of the Click reaction, and derivatizing the 1 position of the triazole.\textsuperscript{[8]}

The Cu\textsuperscript{I} catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes to afford 1,2,3-triazoles has become popular in synthesis.\textsuperscript{[9]} This reaction is an efficient and rapid way to introduce diversity into a library. In addition, compounds containing 1,2,3-triazoles have shown great biological activity, including antibacterial, anti-HIV, and antiallergenic. One other benefit of the reaction is that the two components, the alkyne and azide, are essentially inert to biological and a variety of organic conditions in synthesis, which allows for the two functionalities to be introduced when convenient and remain unaffected through a number of transformations leading up to the Click reaction.\textsuperscript{[8]} Consequently, the Cu\textsuperscript{I} catalyzed 1,3-dipolar cycloaddition was the main inspiration for the 2-AIT amide library explained herein.

2.2 Synthesis of the 2-Aminoimidazole Triazole Amide (2-AIT amide) Library

The first building block of the Click reaction that was synthesized was the boc-protected 2-AI alkyne (Scheme 2.1). Commercially available 5-hexynoic acid was used to create a Boc-protected 2-AI with a 3 methylene unit linker between the 2-AI head group and the alkyne. First 5-hexynoic acid was transformed into the corresponding acid chloride via
treatment with oxalyl chloride and a catalytic amount of DMF. The intermediate underwent homologation via diazomethane, and the resulting α-diazoketone was quenched with hydrobromic acid to afford the α-bromoketone. The α-bromoketone was then condensed with Boc-guanidine to complete the synthesis of the Boc-protected 2-AI alkyne subunit, 2.1.\[^{10}\]

![Scheme 2.1: Synthesis of Boc-protected 2-AI alkyne, 2.1.](image)

There were many advantages in taking this synthetic pathway to the 2-AI alkyne 2.2. One was the ease of purification of the 2-AI alkyne due to the Boc-protecting group. For instance, with the Boc-protecting group intact, a MeOH/NH\(_3\) eluent was avoidable, and instead a simple MeOH/CH\(_2\)Cl\(_2\) eluent was used for chromatographic separation on silica. Also, the high yield of the first three steps was beneficial because it allowed us to bring up large quantities of the α-bromoketone. The only downfall of the pathway was the cyclization step to afford the Boc-protected 2-AI alkyne. On average this step gave a 40% yield.

The use of chloroacetyl chloride as a building block for the azides was explored next (Scheme 2.2). First, commercially available amines were reacted with chloroacetyl chloride to derive a variety of structurally diverse amides. Each amide was then reacted with sodium azide to generate their corresponding azidoacetyl amides (2.3-2.17).
Scheme 2.2: Chloroacetyl chloride as a building block to synthesize azidoacetyl amides.

The synthesis of the azidoacetyl amides only requires 1 chromatographic separation (some compounds are able to be recrystallized), is high yielding, and only takes a day to obtain the final product.

The synthesized azidoacetyl amides were then subjected to the 1,3-dipolar-cycloaddition reaction, otherwise known as the Click reaction, with the Boc-protected 2-AI alkyne, 2.2. This reaction yielded 2-AIT amides 2.18-2.32a, which were then deprotected with TFA followed by ion exchange with HCl to afford the 2-AIT amides as their HCl salts (Scheme 2.3), 2.18-2.32b.
Scheme 2.3: Click reaction and deprotection conditions.

The initial 15 compound library of 2-AIT amides (Figure 2.2) were then ready to be subjected to initial biofilm inhibition screens.
**Figure 2.2:** Initial 15-compound 2-AIT amide library
2.3 Initial Biofilm Inhibition Assays

Each 2-AIT conjugate was initially screened at 400 µM for the ability to modulate biofilm development of *E. coli* and *A. baumannii*. The results of this initial screen are summarized in Table 2.1.

**Table 2.1:** Initial biofilm inhibition screen at 400 µM.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>E.coli</em> Biofilm inhibition at 400 µM (%)</th>
<th><em>A.baumannii</em> (Actb) Biofilm inhibition at 400 µM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.18b</td>
<td>-30.3 ± 7.50</td>
<td>31.4 ± 6.5</td>
</tr>
<tr>
<td>2.19b</td>
<td>90.8 ± 0.52</td>
<td>28.3 ± 6.8</td>
</tr>
<tr>
<td>2.20b</td>
<td>57.8 ± 2.42</td>
<td>36.4 ± 6.1</td>
</tr>
<tr>
<td>2.21b</td>
<td>-127.1 ± 18.7</td>
<td>13.6 ± 3.5</td>
</tr>
<tr>
<td>2.22b</td>
<td>-17.6 ± 4.73</td>
<td>21.2 ± 7.5</td>
</tr>
<tr>
<td>2.23b</td>
<td>-47.8 ± 8.49</td>
<td>15.9 ± 8.0</td>
</tr>
<tr>
<td>2.24b</td>
<td>-169.1 ± 22.1</td>
<td>17.7 ± 3.3</td>
</tr>
<tr>
<td>2.25b</td>
<td>-2.6 ± 5.59</td>
<td>6.2 ± 8.9</td>
</tr>
<tr>
<td>2.26b</td>
<td>-82.7 ± 61.2</td>
<td>0.3 ± 10.8</td>
</tr>
<tr>
<td>2.27b</td>
<td>-141.7 ± 19.9</td>
<td>23.0 ± 3.2</td>
</tr>
<tr>
<td>2.28b</td>
<td>-277.8 ± 31.1</td>
<td>19.5 ± 3.3</td>
</tr>
<tr>
<td>2.29b</td>
<td>-41.8 ± 30.7</td>
<td>0.8 ± 12.3</td>
</tr>
<tr>
<td>2.30b</td>
<td>-169.2 ± 22.1</td>
<td>13.9 ± 3.5</td>
</tr>
<tr>
<td>2.31b</td>
<td>-19.9 ± 6.89</td>
<td>17.5 ± 7.8</td>
</tr>
<tr>
<td>2.32b</td>
<td>-321.2 ± 34.6</td>
<td>8.5 ± 3.7</td>
</tr>
</tbody>
</table>

As can be seen, we were able to rapidly identify a number of compounds that not only selectively inhibited *E. coli* biofilms (in comparison to *A. baumannii*) but also identified a number of compounds that promoted *E. coli* biofilm growth. Compounds 2.28b and 2.32b
promoted *E. coli* biofilm growth by 277% and 321% respectively, while on the other hand 2.19b inhibited biofilm growth by 90%. These results conclude that for *E. coli* inhibition the alky chain off of the amide bond should be greater than a hexyl chain. The conjugates with aryl groups off of the amide promoted biofilm growth ranging from 19% to 321%.

### 2.4 SAR Studies on Lead Compound

With these initial results, an SAR study was performed in an attempt to increase inhibition of both *E. coli* and *A. baumannii* biofilms. The first change we made was to elongate the alky chain between the 2-AI head group and the triazole from 3 to 5 methylene units. This decision was based on the fact that the lead compound of the group, SPAR, contains a 5 methylene spacer between the 2-AI and the triazole (Figure 2.3).[4]

![Figure 2.3](image)

**Figure 2.3:** Modification of spacer length based on lead compound SPAR.

Unfortunately, 7-octynoic acid is not commercially available, so in order to synthesize the new 2-AI alkyne with a 5-methylene unit spacer a longer synthesis was required. Instead, the commercially available internal alkyne, 3-Octyn-1-ol was used as the
starting material for the synthesis of the new 2-Alkyl alkyne. The internal alkyne was isomerized to obtain the terminal alkyne, 7-Octyn-1-ol, and then submitted to the Jones oxidation to achieve the desired acid. Once the acid was synthesized, it underwent homologation via diazomethane, quench, and cyclization with Boc-guanidine to complete the synthesis of the new 2-Alkyl alkyne (Scheme 2.4).

Scheme 2.4: Synthesis of new boc-protected 2-Alkyl alkyne, 2.33.

Azide 2.4 underwent the Click reaction to compound 2.33 since it showed the greatest inhibition against \( E. \ coli \) in the initial screens. Two other azidoacetyl amides, 2.12 and 2.14 were subjected to the cycloaddition with alkyne 2.33 (Scheme 2.5) in hopes to improve biofilm inhibition activity.
Scheme 2.5: Cycloaddition reaction of 2.33 with azidoacetyl amides.

The three new 2-AIT amides were then subjected to screens against both *E. coli* and *A. baumannii* at 400 µM. The results (Table 2.2) of the initial screens did not show an increase in the inhibition of biofilms. However, just like the original 16 conjugates, the new compounds showed selective activity (both inhibition and promotion) towards *E. coli*. By increasing the methylene units between the 2-AI head group and the triazole, the biofilm inhibition did not increase for compound 2.34b; in fact it showed similar inhibition results as compound 2.20b. The elongation of the spacer did not change the activity from promotion to inhibition of biofilms.

Table 2.2: Biofilm inhibition of compounds 2.34-2.36b at 400 µM.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>E. coli</em> Biofilm Inhibition at 400µM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.34b</td>
<td>58.9 ± 8.8</td>
</tr>
<tr>
<td>2.35b</td>
<td>-74.1 ± 59.3</td>
</tr>
<tr>
<td>2.36b</td>
<td>-142.9 ± 52.5</td>
</tr>
</tbody>
</table>
Next, in a second attempt to increase activity, we focused on the tail of the current lead compound in our group, SPAR (Figure 2.4).

**Figure 2.4**: SPAR as an inspiration for a new tail group.
The SPAR tail is synthesized via a commercially available alcohol, trans-2-methyl-3-phenyl-2-propen-1-ol, via a mesylation of the alcohol and then displacement of the mesylate with sodium azide.\(^4\) The resulting azide can then undergo the Staudinger reduction to achieve the necessary amine, and then reacted with chloroacetyl chloride to obtain the desired amide bond (Scheme 2.6).

**Scheme 2.6**: Synthesis of SPAR inspired tail group, 2.38.
Once the desired azide, 2.38, was synthesized, it was also subjected to the cycloaddition reaction with 2.33 and subsequent deprotection to afford 2-AIT amide 2.39b (Scheme 2.7).
Scheme 2.7: Click of SPAR like amide.

Compound 2.39b was then screened for biofilm inhibition against *E. coli* and *A. baumannii* at 400 µM. With the addition of the amide into the tail group (compared to SPAR), all activity dropped. 2.39b showed no activity against *A. baumannii* and promoted *E. coli* biofilms by 76.5%.

One last attempt was made to increase the biofilm inhibition activity. This time, the lead 2-AIT amide compound was used as inspiration. The current lead compound, 2.19b, had a 3 methylene unit spacer between the 2-AI and the triazole and a decyl chain off of the amide. When the same decyl tail was clicked with the 5-methylene unit spacer, 2-AI alkyne activity did not increase, so it was decided to shorten the hydrocarbon tail from decyl to an octyl chain. This change would make the new compound (2.41b) the same length as the current lead (Figure 2.5).
Commercially available octylamine was used to make the azidoacetylamide (2.40), then clicked and deprotected to arrive at compound 2.41b (Figure 2.6). Compound 2.41b was then screened against *E. coli* initially at 400 µM and inhibited biofilms by 95.5 ± 0.7%.

**Figure 2.6: Synthesis of SAR 2-AIT amide 2.41b.**

**2.5 Lead Compound Biological Screens**

For each compound that inhibited *E. coli* biofilm development by >90% a dose response curve was performed to determine their respective IC$_{50}$ values. Both compounds 2.19b and 2.41b fell into this category, and their IC$_{50}$ values were 36.9 ± 1.01% and 13.0 ± 1.01%, respectively. Growth curves were performed on active compounds to determine if the compounds were toxic to the bacteria. Both compounds 2.19b and 2.41b were shown to be non-toxic to *E. coli* at their respective IC$_{50}$ values, which revealed that inhibition of biofilm development was not due to microbicidal activity.
Since it was clear that compounds 2.19b and 2.41b can inhibit biofilm formation of E. coli, their ability to disperse preformed biofilms was tested. The dispersion assays concluded that compound 2.19b was able to disperse preformed E. coli biofilms while on the other hand compound 2.41b had no dispersal activity. A dose response curve was completed for compound 2.19b and it had an EC$_{50}$ value (concentration of compound necessary to elicit 50% biofilm dispersion) of 120.4 ± 12.72 µM. Since 2-AIT amide 2.19b showed both inhibition and dispersion against E. coli biofilms, an additional screen was set up to determine if compound 2.19b would inhibit E. coli biofilm growth on a medical catheter due to the high occurrence of infections associated with them.$^{[12,13,14]}$ The catheter was treated at a concentration of 80 µM of 2.19b because the growth curve showed that the compound was not toxic at that high of a concentration and through visualization of the stained catheter, 2.19b showed to inhibit biofilm growth on a medical catheter, which can be seen in the appendix.

Lastly, red blood cell hemolysis of 2.19b, 2.41b, 2.27b, and 2.32b was performed with defibrinated sheep’s blood. Compounds 2.19b and 2.41b were chosen because these compounds had the greatest inhibition activity against E. coli. On the other hand, compounds 2.27b and 2.32b exhibited the greatest biofilm promotion of E. coli and growth curves on these compounds at 200 µM displayed that they were non-toxic. The HD$_{50}$ (the hemolytic dose that lyses 50% of the red blood cells) for 2.19b and 2.41b was found to be 391 µM and 675 µM, respectively. Both compounds 2.27b and 2.32b had an HD$_{50}$ > 800 µM (which is the highest concentration that was tested).
Table 2.3: Summarized results for 2-AIT amide library.

<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli IC$_{50}$ (µM)$^{a}$</th>
<th>E. coli EC$_{50}$ (µM)$^{a}$</th>
<th>HD$_{50}$ (µM)$^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.19b</td>
<td>36.9 ± 1.02</td>
<td>120.4 ± 12.72</td>
<td>391.54 ± 86.96</td>
</tr>
<tr>
<td>2.41b</td>
<td>3.01 ± 1.01</td>
<td>-</td>
<td>675.11 ± 21.60</td>
</tr>
<tr>
<td>2.27b</td>
<td>-</td>
<td>-</td>
<td>&gt; 800</td>
</tr>
<tr>
<td>2.32b</td>
<td>-</td>
<td>-</td>
<td>&gt; 800</td>
</tr>
</tbody>
</table>

2.6 Conclusion

In conclusion, a library of 2-AIT amides was synthesized in an attempt to target activity against a specific bacterium. In this experiment, the compounds were initially screened against both E. coli and A. baumannii at 400 µM. The 2-AIT amides showed selective inhibition and promotion of E. coli biofilms over A. baumannii. Compounds 2.19b and 2.41b, which were the most active inhibitors of E. coli biofilms, both contained hydrocarbon chains off of the triazole amide, and showed that as the methylene spacer between the 2-AI head and triazole increases from 3 to 5 activity also increases. On the other hand, only compound 2.19b was able to disperse preformed E. coli biofilms with an EC$_{50}$ of 120.4 ± 12.72. Since compound 2.19b was able to both inhibit and disperse biofilms in a 96 well plate, it was tested for its ability to inhibit biofilm formation on a catheter. Compound 2.19b showed inhibition of biofilm growth on the catheters at a concentration of 80 µM compared to the control catheter. Lastly, red blood cell lysis experiments showed that the lead compounds lysed the red blood cells well above their respective IC$_{50}$ and EC$_{50}$ values. Compounds 2.27b and 2.32b, which were the lead promoters of E. coli biofilms, also were
not toxic to the red blood cells, and in fact had an HD$_{50}$ well above the highest concentration tested.

2.7 Experimental

All reagents used for chemical synthesis were purchased from commercially available sources and used without further purification. Chromatography was performed using 60 mesh standard silica gel from Sorbtech. NMR solvents were obtained from Cambridge Isotope Labs and were used as is. $^1$HNMR (300 MHz or 400 MHz) and $^{13}$C NMR (75 MHz or 100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the residual protonated peak of the respective NMR solvents; coupling constants (J) are in hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd= doublet of doublets, t = triplet, dt = doublet of triplets, bt = broad triplet, q = quartet, m = multiplet, bm = broad multiplet. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. Funding was obtained from NCSU Department of Chemistry.

\[
\begin{align*}
\text{Br} & \quad \text{O} \\
\text{C} & \quad \text{C} \\
\text{C} & \quad \text{C} \\
\text{C} & \quad \text{C} \\
\end{align*}
\]

2.1

1-bromohept-6-yn-2-one (2.1): A solution of 5-hexynoic acid (1.23 g, 11.0 mmol) in anhydrous dichloromethane (20 mL) was cooled to 0 °C. Oxalyl chloride (4.2 g, 33.0 mmol) was added drop-wise followed by addition of a catalytic amount of anhydrous DMF (0.01 mL). The reaction was allowed to warm to room temperature over the course of 1 h and after
that time the solution was evaporated to dryness. The crude acid chloride was dissolved in anhydrous dichloromethane (10 mL) and added drop-wise to a 0 °C solution of CH₂N₂ (33.0 mmol generated from Diazald®/KOH) in Et₂O (100 mL). This solution was stirred at 0 °C for 1 h upon which the reaction was quenched via the drop-wise addition of a 48% solution of conc. HBr (4.0 mL). The reaction mixture was diluted with dichloromethane (15 mL) and immediately washed with sat. NaHCO₃ (3 x 25 mL) and brine (2 x 25 mL) before being dried (Na₂SO₄), filtered and concentrated. The crude oil was purified via flash column chromatography (100% DCM) to obtain 1-bromohept-6-yn-2-one 2.1 (2.01 g, 97%) as a pale yellow oil: ¹H NMR (300 MHz, CDCl₃) δ 3.89 (s, 2H), 2.80 (t, 2H, J = 6.9 Hz), 2.24 (dt, 2H, J = 6.9, 2.4 Hz), 1.97 (t, 1H, J = 2.4 Hz), 1.85 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 201.7, 83.3, 69.6, 38.4, 34.5, 22.5, 17.7; HRMS (ESI) calced for C₇H₉BrO (M⁺) 188.9910, found 188.9914.

\[
\text{H₂N} \quad \text{Boc}
\]

**2.2 tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate (2.2):** To a solution of 1-bromohept-6-yn-2-one 2.1 (2.01 g, 10.6 mmol) in anhydrous DMF (30 mL) was added Boc-guanidine (5.20 g, 32.7 mmol). The reaction was stirred at ambient temperature for 72 h upon which the mixture was evaporated to dryness. The solid was partitioned between EtOAc (150 mL) and water (75 mL). The organic layer was successively washed with water (3 x 50 mL) and brine (2 x 50 mL) before being dried (Na₂SO₄) and evaporated to dryness.
The resulting crude oil was purified via flash column chromatography (1-10% MeOH/DCM) to obtain tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate 2.2 (1.07 g, 40%) as a yellow solid: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.49 (s, 1H), 2.43 (t, 2H, $J = 7.2$ Hz), 2.18 (dt, 2H, $J = 3$, 7.2 Hz), 1.93 (t, 1H, $J = 1.4$ Hz), 1.78 (m, 2H), 1.54 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.8, 149.3, 137.8, 106.5, 84.4, 84.3, 68.6, 28.2, 28.0, 27.2, 26.9, 17.8; HRMS (ESI) calcd for C$_{13}$H$_{19}$N$_3$O$_2$ (M$^+$) 249.14763, found 249.14773.

![2.3](image)

2-azido-N-hexylacetamide (2.3): Compound provided by Dr. Robert Huigens III

![2.4](image)

2-azido-N-decylacetamide (2.4): To a solution of decylamine (2.10 g, 13.6 mmol) in DCM (20 mL) was added triethyl amine (2.20 mL, 16.1 mmol). The reaction was stirred at 0 °C for 5 minutes then chloroacetyl chloride (1.40 g, 12.4 mmol) was added to the reaction dropwise. The reaction was stirred at 0 °C for 20 minutes and then allowed to warm to room temperature for an additional 45 minutes. The reaction was quenched with 5% HCl and then extracted with EtOAc (2 x 50 mL). The organic layer was dried (Na$_2$SO$_4$) and evaporated to dryness. The crude solid (1.10 g, 4.7 mmol) was dissolved in acetone (20 mL) and water (10
mL) and sodium azide (1.50 g, 23.5 mmol) was added to the reaction. The reaction was refluxed for 24 h, quenched with brine (20 mL) and extracted with EtOAc (50 mL). The organic layer was dried (Na$_2$SO$_4$) and evaporated to dryness. The crude solid was purified via recrystallization in hexanes to afford the title compound (3.10 g, 97%) as a brown solid:

$^1$H NMR (300 MHz, CDCl$_3$) δ 6.30 (s, 1H), 3.97 (s, 2H), 3.27 (q, 2H, $J = 7.2$ Hz), 1.50 (m, 2H), 1.28 (bs, 14H), 0.87 (t, 3H, 6.6 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.9, 52.9, 39.7, 32.0, 29.7, 29.6, 29.5, 29.5, 27.0, 22.8, 14.3; HRMS (ESI) calcd for C$_{12}$H$_{25}$N$_4$O (M$^+$) 240.1950, found 240.1949.

![](image1.png)

2-azido-$N$-tetradecylacetamide (2.5): Following the same procedure as 2-azido-$N$-hexylacetamide (2.3), 0.710 g (3.33 mmol) of tetradecylamine afforded 0.683 g (65%) of the title compound as a tan solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 6.33 (bs, 1H), 3.97 (s, 2H), 3.26 (q, 2H, $J = 6.8$ Hz), 1.51 (m, 2H), 1.24 (m, 22H), 0.86 (t, 3H, $J = 6.8$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 166.6, 52.9, 39.7, 32.1, 29.9, 29.8 (3), 29.7 (2), 29.6, 29.5, 29.4, 27.0, 14.3; HRMS (ESI) calcd for C$_{16}$H$_{32}$N$_4$O (M$^+$) 296.2576, found 296.2577.

![](image2.png)
2-azido-N-isopropylacetamide (2.6): Compound synthesized by Dr. Steven Rogers.

\[ \text{Structure of 2-azido-N-isopropylacetamide} \]

2-azido-N-tert-butylacetamide (2.7): Compound synthesized by Dr. Steven Rogers.

\[ \text{Structure of 2-azido-N-tert-butylacetamide} \]

2-azido-N-cyclopentylacetamide (2.8): Compound synthesized by Dr. Steven Rogers.

\[ \text{Structure of 2-azido-N-cyclopentylacetamide} \]

2-azido-N-cyclohexylacetamide (2.9): Compound synthesized by Dr. Robert Huigens III.

\[ \text{Structure of 2-azido-N-cyclohexylacetamide} \]

2-azido-1-(pyrrolidin-1-yl)ethanone (2.10): Compound synthesized by Dr. Steven Rogers.

\[ \text{Structure of 2-azido-1-(pyrrolidin-1-yl)ethanone} \]
2-azido-1-morpholinoethanone (2.11): Compound synthesized by Dr. Robert Huigens III.

\[
\begin{array}{c}
\text{O} \\
\text{N}_3 \\
\text{N}
\end{array}
\]

\text{2.12}

2-azido-N-phenylacetamide (2.12): Compound synthesized by Dr. Robert Huigens III.

\[
\begin{array}{c}
\text{O} \\
\text{N}_3 \\
\text{N}
\end{array}
\]

\text{2.13}

2-azido-N-benzylacetamide (2.13): Following the same procedure as 2-azido-N-decylacetamide (2.4), 1.50 g (13.6 mmol) of benzylamine afforded 1.02 g (78%) of the title compound as a brown solid: \(^1\text{H NMR} (300 \text{ MHz, CDCl}_3) \delta 7.30 (\text{m, 5H}), 6.98 (\text{bs, 1H}), 4.40 (\text{s, 2H}), 3.86 (\text{d, 2H, } J = 6.9 \text{ Hz}); \ ^{13}\text{C NMR} (75 \text{ MHz, CDCl}_3) \delta 166.8, 137.6, 128.8, 127.9, 127.8, 52.5, 43.5; \text{HMRS (ESI) calcd for } C_9H_{10}N_4O (M^+) 191.0927, \text{ found 191.0928.}

\[
\begin{array}{c}
\text{O} \\
\text{N}_3 \\
\text{N}
\end{array}
\]

\text{2.14}

2-azido-N-phenethylacetamide (2.14): Compound synthesized by Dr. Robert Huigens III.
2-azido-N-(4-methoxyphenyl)acetamide (2.15): Compound synthesized by Dr. Robert Huigens III.

2-azido-N-(4-methoxybenzyl)acetamide (2.16): Compound synthesized by Dr. Robert Huigens III.

2-azido-N-(4-methoxyphenethyl)acetamide (2.17): Compound synthesized by Dr. Robert Huigens III.

**General Procedure for Click Reaction and Subsequent Boc Deprotection:** The terminal alkyne (1.0 equiv.) and the appropriate azide (3.0 equiv.) were dissolved in a 1:1:1 mixture of ethanol, water, and dichloromethane (ca. 3.6 mL per 0.100 g of terminal alkyne). To this
solution sodium ascorbate (60 mol%) and copper (II) sulfate pentahydrate (30 mol%) were added while stirring at room temperature. Reaction mixtures were allowed to stir until completion via TLC analysis (12-24 hrs). The solvents were then removed in vacuo, after which the resulting residue was dissolved in dichloromethane and purified via silica gel column chromatography (1:40 methanol: dichloromethane to 1:10 methanol: dichloromethane). To remove the Boc protecting group, the resulting product was then dissolved in a 1:4 trifluoroacetic acid: dichloromethane mixture and allowed to stir for 1.5 hours. Upon completion, the reaction mixture was concentrated in vacuo and then left on a high vacuum overnight. Then, methanol supplemented with HCl was added to the product forming the HCl salt of the deprotected product and then concentrated in vacuo. The resulting residue was washed with diethyl ether and then placed on a high vacuum overnight.

2.18b

2-{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl}-N-hexyl-acetamide hydrochloride (2.18b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-hexylacetamide, 2.3 (0.221 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(hexylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.18a:

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.52 (bs, 1H), 7.18 (bs, 1H), 6.46 (s, 1H), 6.20 (bs, 1H), 4.98 (s, 2H), 3.13 (s, 2H), 2.67 (bs, 2H), 2.34 (bs, 2H), 1.88 (bs, 2H), 1.50 (bs, 9H), 1.16 (bs, 8H),
0.77 (t, 3H, \( J = 6.6 \) Hz); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 165.6, 150.6, 149.3, 148.2, 137.8, 122.9, 106.6, 99.9, 84.8, 52.8, 39.9, 31.4, 29.7, 29.2, 28.2, 28.0, 27.4, 26.5, 25.0, 22.5, 14.0; HRMS (ESI) calcd for C\(_{12}\)H\(_{35}\)N\(_7\)O\(_3\) (\( M^+ \)) 433.2802, found 433.2803. Following the general deprotection procedure tert-butyl 2-amino-4-(3-(1-(2-(hexylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, \( 2.18a \) (0.073 g, 0.17 mmol) afforded 0.061 g (78%) of the title compound as a yellow solid: \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) 8.46 (s, 1H), 6.59 (s, 1H), 5.25 (s, 2H), 3.23 (s, 1H), 2.84 (bs, 2H), 2.58 (bs, 2H), 2.03 (bs, 2H), 1.53 (t, 2H, \( J = 11.2 \) Hz), 1.30 (m, 8H), 0.89 (t, 3H, \( J = 6.6 \) Hz); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \( \delta \) 175.8, 167.2, 148.6, 128.2, 110.3, 54.2, 40.9, 32.7, 30.3, 28.6, 27.8, 25.0, 24.8, 23.7, 14.5; HRMS (ESI) calcd for C\(_{16}\)H\(_{27}\)N\(_7\)O (\( M^+ \)) 333.2277, found 333.2271.

\[ \text{2.19b} \]

2-{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-1H-1,2,3-triazol-1-yl}-N-decyl-acetamide hydrochloride (\( 2.19b \)):

Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, \( 2.2 \) (0.100 g, 0.40 mmol) was reacted with 2-azido-N-decylacetamide, \( 2.4 \) (0.288 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2- (decylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, \( 2.19a \):

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.48 (s, 1H), 6.53 (s, 1H), 5.00 (s, 2H), 3.21 (d, 2H, \( J = 6.3 \) Hz), 2.77 (bs, 2H), 2.42 (bs, 2H), 1.97 (bs, 2H), 1.57 (s, 1H), 1.23 (s, 16H), 0.86 (t, 3H, \( J = 6.6 \) Hz); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 168.4, 167.7, 156.1, 152.3, 142.7, 134.3, 122.7,
HRMS (ESI) calcd for C\textsubscript{25}H\textsubscript{34}N\textsubscript{7}O\textsubscript{3} (M\textsuperscript{+}) 489.3427, found 489.3424. Following the general deprotection procedure \textit{tert}-butyl 2-amino-4-(3-(1-(2-decylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, \textbf{2.19a} afforded 0.055g (59\%) of the title compound as a tan solid: \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD) δ 8.26 (s, 1H), 6.56 (s, 1H), 5.30 (s, 2H), 3.23 (t, 2H, \textit{J} = 7.6 Hz), 2.87 (bs, 2H), 2.59 (bs, 2H), 2.03 (bs, 2H), 1.54 (t, 2H, \textit{J} = 6.8 Hz), 1.32-1.29 (m, 16H), 0.89 (t, 3H, \textit{J} = 6.6 Hz); \textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD) δ 184.1, 166.9, 148.9, 148.6, 128.0, 110.3, 54.4, 40.9, 33.2, 30.8, 30.6, 30.5, 30.4, 28.5, 28.1, 24.8, 24.6, 23.8, 14.6; HRMS (ESI) calcd for C\textsubscript{20}H\textsubscript{35}N\textsubscript{7}O (M\textsuperscript{+}) 389.2903, found 389.2900.

\textbf{2.20b}  

\textbf{2-{[4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-tetradecyl-acetamide hydrochloride (2.20b):} Following the general Click procedure, \textit{tert}-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, \textbf{2.2} (0.100 g, 0.40 mmol) was reacted with 2-azido-N-tetradecylacetamide, \textbf{2.5} (0.356 g, 1.20 mmol) affording \textit{tert}-butyl 2-amino-4-(3-(1-(2-oxo-2-(tetradecylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, \textbf{2.20a}: \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.51 (s, 1H), 6.52 (s, 1H), 5.00 (s, 2H), 3.20 (d, 2H, \textit{J} = 6.6 Hz), 2.76 (t, 2H, \textit{J} = 6.9 Hz), 2.42 (bs, 2H), 1.96 (bs, 2H), 1.55 (s, 9H), 1.24 (s, 24H), 0.87 (t, 3H, \textit{J} = 6.6 Hz); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 184.1, 166.9, 148.9, 148.6, 128.0, 110.3, 54.4, 40.9, 33.2, 30.8, 30.6, 30.5, 30.4, 28.5, 28.1, 24.8, 24.6, 23.8, 14.6; HRMS (ESI) calcd for C\textsubscript{29}H\textsubscript{51}N\textsubscript{7}O\textsubscript{3} (M\textsuperscript{+}) 545.4053, found 545.4045. Following the general deprotection procedure 0.067 g (0.12
mmol) of tert-butyl 2-amino-4-(3-(1-(2-oxo-2-(tetradecylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.20a afforded 0.059 g (70%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.40 (s, 1H), 6.59 (s, 1H), 5.37 (s, 2H), 3.23 (t, 1H, $J$ = 6.8 Hz), 2.92 (bs, 2H), 2.613 (t, 2H, $J$ = 6.8 Hz), 2.05 (bs, 2H), 1.54 (t, 2H, $J$ = 6.4 Hz), 1.28 (s, 24H), 0.89 (t, 3H, $J$ = 6.8 Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 181.1, 166.3, 148.6, 140.1, 127.8, 110.4, 54.9, 40.9, 33.2, 30.9, 30.8, 30.6, 30.5, 30.4, 28.2, 28.1, 24.7, 24.1, 23.8, 14.6; HRMS (ESI) calcd for C$_{24}$H$_{43}$N$_7$O (M$^+$) 445.3529, found 445.3519.

$^2$-{4-[3-(2-amino-1H-imidazol-4-yl)propyl]-[1,2,3]triazol-1-yl]-N-isopropyl-acetamide hydrochloride (2.21b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-isopropylacetamide, 2.6 (0.170 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(isopropylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.21a: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.52 (s, 1H), 6.44 (s, 1H), 4.94 (s, 2H), 3.93 (m, 1H), 2.65 (bs, 2H), 2.33 (bs, 2H), 1.88 (bs, 2H), 1.49 (s, 9H), 1.02 (d, 6H, $J$ = 6.8 Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 164.8, 150.5, 149.3, 148.0, 137.6, 123.0, 106.6, 84.8, 52.7, 42.0, 28.3, 28.1, 27.9, 27.3, 25.0, 22.3; HRMS (ESI) calcd for C$_{18}$H$_{29}$N$_7$O$_3$ (M$^+$) 391.2332, found 391.2332. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(isopropylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate,
2.21a (0.089 g, 0.23 mmol) afforded 0.074 g (57%) of the title compound as a yellow solid:

$^1$H NMR (400 MHz, CD$_3$OD) δ 8.27 (s, 1H), 6.58 (s, 1H), 5.30 (s, 2H), 3.99 (s, 1H), 3.35 (s, 1H), 2.88 (t, 2H, $J = 6.4$ Hz), 2.59 (t, 2H, $J = 7.2$ Hz), 2.05-2.01 (m, 2H), 1.18 (d, 6H, $J = 6.8$ Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 165.8, 148.6, 127.9, 110.3, 54.5, 43.5, 28.4, 24.8, 24.4, 22.6; HRMS (ESI) calcd for C$_{13}$H$_{21}$N$_7$O (M$^+$) 291.1807, found 291.1804.

2.22b

2-\{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl\}-N-\{tert-butyl\}-acetamide hydrochloride (2.22b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-\{tert-butyl\}acetamide, 2.7 (0.187 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(\{tert-butylamino\})-2-oxoethyl)-1H,1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.22a:

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.50 (s, 1H), 6.53 (s, 1H), 6.05 (bs, 1H), 4.92 (s, 2H), 2.77 (t, 2H, $J = 7.2$ Hz), 2.43 (bs, 2H), 1.98 (bs, 2H), 1.58 (s, 9H), 1.30 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 164.8, 150.5, 149.4, 148.2, 137.5, 123.0, 106.9, 85.1, 53.4, 52.0, 28.6, 28.2, 28.0, 25.1; HRMS (ESI) calcd for C$_{19}$H$_{31}$N$_7$O$_3$ (M$^+$) 405.2488, found 405.2489. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(\{tert-butylamino\})-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.22a (0.063 g, 0.16 mmol) afforded 0.055 g (80%) of the title compound as a yellow solid: $^1$H NMR (400 MHz,
CD$_3$OD) δ 6.57 (s, 1H), 5.23 (s, 2H), 2.85 (bs, 2H), 2.59 (m, 2H), 2.04 (bs, 2H), 1.35 (s, 9H);

$^{13}$C NMR (100 MHz, CD$_3$OD) δ 166.1, 148.6, 128.1, 110.3, 66.1, 54.8, 52.8, 28.9, 28.5, 24.8, 15.6; HRMS (ESI) calcd for C$_{14}$H$_{23}$N$_7$O ($M^+$) 305.1964, found 305.1959.

2.23b

2-\{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-cyclopentyl-acetamide hydrochloride (2.23b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-cyclopentylacetamide, 2.8 (0.202 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(cyclopentylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate,

2.23a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.51 (s, 1H), 6.52 (s, 1H), 4.98 (s, 2H), 4.14 (m, 2H), 2.77 (bs, 2H), 2.42 (bs, 2H), 1.94 (bs, 6H), 1.57 (s, 9H), 1.32 (m, 2H), 1.24 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 166.0, 149.9, 149.4, 148.4, 137.5, 122.9, 106.9, 85.1, 53.1, 51.7, 32.9, 29.9, 28.1, 25.2, 23.8; HRMS (ESI) calcd for C$_{20}$H$_{32}$N$_7$O$_3$ ($M^+$) 417.2488, found 417.2488.

Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(cyclopentylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate,

2.23a (0.054 g, 0.13 mmol) afforded 0.045 g (59%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) δ 8.23 (s, 1H), 6.57 (s, 1H), 5.29 (s, 2H), 3.98 (s, 1H), 3.35 (s, 1H), 2.87 (t, 2H, $J$ = 6.4 Hz), 2.59 (t, 2H, $J$ = 6.8 Hz), 2.02 (t, 2H, $J$ = 6.8 Hz), 1.94-1.89 (m,
2H), 1.75 (bs, 2H), 1.61 (t, 2H, J = 6.8 Hz), 1.57-1.51 (m, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 166.3, 148.6, 128.0, 110.3, 55.4, 54.4, 52.9, 33.6, 28.4, 24.9, 24.7, 24.5; HRMS (ESI) calcd for C$_{15}$H$_{23}$N$_7$O (M$^+$) 317.1964, found 317.1961.

2-{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl}-N-cyclohexyl-acetamide hydrochloride (2.24b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-cyclohexylacetamide, 2.9 (0.219 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(cyclohexylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.24a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.51 (bs, 1H), 6.43 (bs, 1H), 4.94 (s, 2H), 3.57 (d, 2H, J = 13.2 Hz), 2.64 (bs, 2H), 1.73 (bs, 2H), 1.48 (s, 9H), 1.04 (bs, 10H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 164.6, 158.9, 150.6, 149.3, 148.0, 147.1, 137.5, 122.9, 106.4, 84.8, 52.7, 48.8, 32.5, 28.1, 28.0, 27.9, 25.3, 25.0, 24.7; HRMS (ESI) calcd for C$_{21}$H$_{33}$N$_7$O$_3$ (M$^+$) 431.2645, found 431.2643. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(cyclohexylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.24a (0.075 g, 0.17 mmol) afforded 0.062 g (70%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) δ 8.59 (s, 1H), 6.61 (s, 1H), 5.42 (s, 2H), 3.65 (s, 1H), 3.26 (s, 1H), 2.97 (bs, 2H), 2.63 (bs, 2H), 2.08 (bs, 2H), 1.87 (m, 2H), 1.75 (bs, 2H), 1.38-1.18 (m, 6H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 164.9, 148.6, 130.4, 127.7, 110.5, 55.4, 50.5, 33.6,
28.0, 26.6, 26.0, 24.7, 23.8; HRMS (ESI) calcd for C_{16}H_{26}N_{7}O (M^{+}) 331.2120, found 331.2114.

2.25b

2-[(4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-1-pyrrolidin-1-yl-ethanone hydrochloride (2.25): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.20 mmol) was reacted with 2-azido-1-(pyrrolidin-1-yl)ethanone 2.10 (0.093 g, 0.60 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.25a: ¹H NMR (300 MHz, CDCl₃) δ 7.56 (s, 1H), 6.53 (bs, 1H), 5.80 (bs, 2H), 5.10 (s, 2H), 3.50 (m, 4H), 2.78 (bs, 2H), 2.43 (bs, 2H), 1.99 (m, 2H) 1.88 (m, 4H), 1.57 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 163.7, 151.2, 149.4, 148.2, 122.7, 106.9, 85.2, 70.7, 51.9, 46.5, 29.9, 28.3, 27.9, 27.5, 26.3, 25.3, 24.3; HRMS (ESI) calcd for C_{19}H_{29}N_{7}O_{3} (M^{+}) 403.2332, found 403.2330. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.25a (0.044 g, 0.11 mmol) afforded 0.037 g (84%) of the title compound as a yellow solid: ¹H NMR (400 MHz, CD₃OD) δ 8.54 (s, 1H), 6.60 (s, 1H), 5.69 (s, 2H), 3.64 (t, 2H, J = 6.4 Hz), 3.45 (t, 2H, J = 6.8 Hz), 3.35 (s, 1H), 2.97 (bs, 2H), 2.63 (bs, 2H), 2.07 (t, 4H, J = 6.8 Hz), 1.97-1.92 (m, 2H); ¹³C NMR (100MHz, CD₃OD) δ 164.4, 148.7, 127.7,
110.5, 55.3, 47.7, 47.3, 28.0, 27.1, 24.7, 23.8; HRMS (ESI) calcd for C_{14}H_{21}N_{7}O (M^+) 303.1807, found 303.1809.

2-{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-morpholin-4-yl-acetamide hydrochloride (2.26b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-1-morpholinoethanone, 2.11 (0.204 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-morpholino-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.26a: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.57 (s, 1H), 6.51 (s, 1H), 5.19 (s, 2H), 3.63 (bs, 4H), 3.56 (m, 4H), 2.72 (bs, 2H), 2.39 (bs, 2H), 1.92 (bs, 2H), 1.54 (s, 9H); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 164.2, 149.3, 148.2, 147.2, 137.4, 127.8, 106.7, 85.1, 66.6, 66.4, 50.6, 45.8, 42.6, 29.8, 28.0, 25.2, 24.7; HRMS (ESI) calcd for C_{19}H_{29}N_{7}O_{4} (M^+) 419.2281, found 419.2274. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-morpholino-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.26a (0.062 g, 0.15 mmol) afforded 0.050 g (77%) of the title compound as a green solid: \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 8.58 (s, 1H), 6.61 (s, 1H), 5.85 (s, 2H), 3.76 (bs, 2H), 3.7 (bs, 2H), 3.61 (m, 4H), 3.34 (s, 1H), 2.89 (bs, 2H), 2.64 (m, 2H), 2.09 (bs, 2H); \(^13\)C NMR (100
60 MHz, CD$_3$OD) δ 164.7, 148.7, 127.6, 110.5, 67.5, 55.1, 46.7, 43.9, 36.6, 27.9, 24.7, 23.7; HRMS (ESI) calcd for C$_{14}$H$_{21}$N$_7$O$_2$ (M$^+$) 319.1756, found 319.1756.

2-{[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-phenyl-acetamide hydrochloride (2.27b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-phenylacetamide, 2.12 (0.211 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-2-oxo-2-(phenylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.27a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.51 (bs, 2H), 7.03 (bs, 3H), 6.51 (bs, 1H), 5.25 (bs, 2H), 2.67 (bs, 2H), 2.44 (bs, 2H), 1.93 (bs, 2H), 1.56 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 164.2, 156.2, 149.6, 149.2, 148.5, 145.9, 137.8, 129.0, 124.7, 123.5, 120.3, 106.9, 85.9, 53.3, 29.8, 28.2, 28.1, 24.6; HRMS (ESI) calcd for C$_{21}$H$_{27}$N$_7$O$_3$ (M$^+$) 425.2175, found 425.2175.

Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-2-oxo-2-(phenylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.27a (0.078 g, 0.18 mmol) afforded 0.064 g (52%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) δ 8.76 (s, 1H), 7.58 (d, 2H, $J = 6.8$ Hz), 7.29 (t, 2H, $J = 6.8$ Hz), 7.09 (t, 1H, $J = 7.2$ Hz), 6.59 (s, 1H), 5.67 (s, 2H), 3.34 (s, 1H), 2.98 (bs, 2H), 2.62 (bs, 2H), 2.09 (bs,
2H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 163.9, 148.6, 139.1, 130.1, 127.6, 125.9, 121.3, 110.6, 56.2, 27.9, 24.3, 23.9; HRMS (ESI) calcd for C$_{16}$H$_{19}$N$_7$O (M$^+$) 325.1651, found 325.1644.

2-\{4-\{3-\{(2-amino-1H-imidazol-4-yl)propyl\}-[1,2,3]triazol-1-yl\}-N-benzyl-acetamide hydrochloride (2.28b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-benzylacetamide, 2.13 (0.228 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(benzylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.28a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.84 (bs, 1H), 7.54 (bs, 1H), 7.20 (bs, 5H), 6.52 (s, 1H), 5.04 (s, 2H), 4.36 (s, 2H), 2.68 (bs, 2H), 2.37 (bs, 2H), 1.79 (bs, 2H), 1.57 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 165.7, 149.4, 149.0, 148.1, 137.7, 128.6, 127.7, 127.5, 122.9, 106.9, 85.0, 52.7, 43.6, 29.7, 28.0, 27.8, 25.0; HRMS (ESI) calcd for C$_{22}$H$_{29}$N$_7$O$_3$ (M$^+$) 439.2332, found 439.2330. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(benzylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.28a (0.073 g, 0.017 mmol) afforded 0.060 g (42%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) δ 8.52 (s, 1H), 7.32-7.25 (m, 5H), 6.59 (s, 1H), 5.48 (s, 2H), 4.43 (s, 2H), 3.34 (s, 1H), 2.94 (bs, 2H), 2.61 (bs, 2H), 2.06 (bs, 2H); $^{13}$C NMR
2-{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-phenethyl-acetamide hydrochloride (2.29b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-phenethylacetamide, 2.14 (0.240 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-oxo-2-(phenethylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.29a:

\[ \delta_{1H}^\text{NMR (300 MHz, CDCl}_3\right] = 7.39 (s, 1H), 7.24 (m, 2H), 7.10 (m, 2H), 6.54 (s, 1H), 6.26 (bs, 1H), 4.96 (s, 2H), 3.48 (d, 2H, J = 6.0 Hz), 2.76 (t, 4H, J = 6.6 Hz), 2.44 (bs, 2H), 1.96 (bs, 2H), 1.58 (s, 9H); \]

\[ \delta_{13C}^\text{NMR (75 MHz, CDCl}_3\right] = 165.7, 149.4, 148.4, 138.5, 128.8, 128.7, 126.6, 122.8, 106.9, 85.1, 52.9, 41.0, 35.4, 29.8, 28.3, 28.1, 27.4, 25.1; \]

HRMS (ESI) calcd for C_{23}H_{31}N_{7}O_{3} (M^+) 453.2488, found 453.2488. Following the general deprotection procedure, affording tert-butyl 2-amino-4-(3-(1-(2-oxo-2-(phenethylamino)ethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.29a (0.074 g, 0.16 mmol) afforded 0.061 g (42%) of the title compound as a yellow solid:

\[ \delta_{1H}^\text{NMR (400 MHz, CD}_3\text{OD)} = 8.67 (s, 1H), 7.34-7.18 (m, 5H), 6.61 (s, 1H), 3.47 (bs, 2H), 3.35 (s, 1H), 3.17 (bs, 2H), 2.99 (t, 2H, J \]
= 8.4 Hz), 2.84 (t, 2H, J = 6.8 Hz), 2.63 (bs, 2H), 2.08 (bs, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 167.3, 165.5, 140.2, 138.1, 130.0, 129.9, 129.6, 128.2, 127.5, 110.5, 55.7, 42.5, 36.4, 34.5, 27.9, 24.7; HRMS (ESI) calcd for C$_{18}$H$_{23}$N$_7$O (M$^+$) 353.1964, found 353.1965.

2-\{4-[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl\}-N-(4-methoxy-phenyl)-acetamide hydrochloride (2.30b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.050 g, 0.20 mmol) was reacted with 2-azido-N-(4-methoxyphenyl)acetamide, 2.15 (0.124 g, 0.60 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(4-methoxyphenylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.30a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.58 (bs, 1H), 7.34 (bs, 2H), 6.72 (bs, 2H), 6.51 (bs, 1H), 5.20 (bs, 2H), 3.62 (s, 3H), 2.67 (bs, 2H), 2.37 (bs, 2H), 1.90 (bs, 2H), 1.50 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 164.0, 156.6, 150.5, 149.3, 148.0, 137.6, 130.8, 123.3, 122.0, 114.0, 106.9, 85.0, 55.4, 53.1, 29.8, 28.2, 28.0, 27.2, 25.0; HRMS (ESI) calcd for C$_{22}$H$_{29}$N$_7$O$_4$ (M$^+$) 455.2281, found 455.2279. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(4-methoxyphenylamino)-2-
oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.30a (0.060 g, 0.13 mmol) afforded 0.054 g (60%) of the title compound as a yellow solid: \( ^1H \) NMR (400 MHz, CD\(_3\)OD) \( \delta \) 8.30 (s, 1H), 7.48 (d, 2H, \( J = 8.8 \) Hz), 6.86 (d, 2H, \( J = 8.8 \) Hz), 6.55 (s, 1H), 5.48 (s, 1H), 3.75 (s, 3H), 3.34 (s, 1H), 2.86 (bs, 2H), 2.57 (bs, 2H), 2.01 (bs, 2H); \( ^{13}C \) NMR (100 MHz, CD\(_3\)OD) \( \delta \) 164.8, 158.3, 148.6, 132.1, 128.0, 123.0, 115.2, 110.3, 56.0, 54.9, 28.4, 24.7, 24.6; HRMS (ESI), calcd for C\(_{17}\)H\(_{21}\)N\(_7\)O\(_2\) (M\(^+\)) 355.1756, found 355.1756.

![Image](image_url)

2.31b

2-{4-[3-(2-amino-1H-imidazol-4-yl)propyl]-1,2,3-triazol-1-yl}-N-(4-methoxy-benzyl)-acetamide hydrochloride (2.31b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.050 g, 0.20 mmol) was reacted with 2-azido-N-(4-methoxybenzyl)acetamide, 2.16 (0.132 g, 0.60 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(4-methoxybenzylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.31a: \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.44 (s, 1H), 7.14 (d, 2H, \( J = 8.7 \) Hz), 6.82 (d, 2H, \( J = 8.7 \) Hz), 6.54 (s, 1H), 6.29 (bs, 1H), 5.023 (s, 2H), 4.35 (d, 2H, \( J = 5.1 \)Hz), 3.78 (s, 3H), 2.78 (bs, 2H), 2.42 (s, 2H), 1.97 (bs, 2H), 1.58 (s, 9H); \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)) \( \delta \); HRMS (ESI) calcd for C\(_{23}\)H\(_{31}\)N\(_7\)O\(_4\) (M\(^+\)) 469.2438, found 469.2436. Following the general deprotection procedure, affording tert-butyl 2-amino-4-(3-
(1-(2-(4-methoxybenzylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, 2.31a (0.065 g, 0.14 mmol) afforded 0.054 g (71%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.52 (s, 1H), 7.24 (d, 2H, $J = 8.4$ Hz), 6.87 (d, 2H, $J = 8.4$ Hz), 6.59 (s, 1H), 5.47 (s, 2H), 4.35 (s, 2H), 3.76 (s, 3H), 3.35 (s, 1H), 2.95 (t, 2H, $J = 7.2$ Hz), 2.62 (t, 2H, $J = 7.2$ Hz), 2.06 (t, 2H, $J = 6.8$ Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 165.9, 160.7, 148.6, 145.6, 131.2, 130.3, 129.5, 127.7, 115.1, 110.4, 55.9, 55.3, 44.1, 28.0, 24.7, 23.8; HRMS (ESI) calcd for C$_{18}$H$_{23}$N$_7$O$_2$ (M$^+$) 369.1913, found 369.1911

2-{[3-(2-amino-1H-imidazol-4-yl)-propyl]-[1,2,3]triazol-1-yl]-N-[2-(4-methoxyphenyl)-ethyl]-acetamide hydrochloride (2.32b): Following the general Click procedure, tert-butyl 2-amino-4-(pent-4-ynyl)-1H-imidazole-1-carboxylate, 2.2 (0.100 g, 0.40 mmol) was reacted with 2-azido-N-(4-methoxyphenethyl)acetamide, 2.17 (0.281 g, 1.20 mmol) affording tert-butyl 2-amino-4-(3-(1-(2-(4-methoxyphenethylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate. 2.32a: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (bs, 1H), 6.97 (d, 2H, $J = 6.9$ Hz), 6.73 (d, 2H, $J = 7.5$ Hz), 6.51 (bs, 1H), 4.95 (s, 2H), 3.70 (s, 3H), 3.39 (bs, 2H), 2.66 (bs, 4H), 2.45 (bs, 2H), 1.90 (bs, 2H), 1.56 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 165.7, 158.3, 150.3, 149.8, 149.5, 148.6, 130.5, 129.7, 122.5, 114.0, 85.0, 55.3, 52.8, 41.2, 34.5, 31.0, 29.7, 28.2, 25.0; HRMS (ESI) calcd for C$_{24}$H$_{33}$N$_7$O$_4$ (M$^+$)
483.2594, found 483.2591. Following the general deprotection procedure, tert-butyl 2-amino-4-(3-(1-(2-(4-methoxyphenethylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)propyl)-1H-imidazole-1-carboxylate, **2.32a** (0.066 g, 0.14 mmol) afforded 0.056 g (61%) of the title compound as a yellow solid: \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 8.29 (s, 1H), 7.12 (d, 2H, \(J = 8.0\) Hz), 6.83 (d, 2H, \(J = 8.4\) Hz), 6.56 (s, 1H), 5.27 (s, 2H), 3.74 (s, 3H), 3.42 (t, 2H, \(J = 7.2\) Hz), 2.86 (bs, 2H), 2.76 (t, 2H, \(J = 6.8\) Hz), 2.59 (bs, 2H), 2.03 (bs, 2H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) 167.1, 159.9, 148.6, 132.2, 130.9, 128.2, 115.1, 110.3, 67.0, 55.9, 54.3, 42.6, 35.6, 28.5, 24.8, 15.6; HRMS (ESI), calcd for C\(_{19}\)H\(_{25}\)N\(_7\)O\(_2\) (M\(^+\)) 383.2069, found 383.2069.

**2.34b**

2-\{4-[5-(2-amino-1H-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl]-N-decyl-acetamide hydrochloride (2.34b): Following the general Click procedure, 2-amino-4-hept-6-ynyl-imidazole-1-carboxylic acid tert-butyl ester, **2.33** (0.075g, 0.270 mmol) was reacted with 2-azido-N-decyl-acetamide, **2.4** (0.097g, 0.405 mmol) affording **2.34a**: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.52 (bs, 1H), 6.47 (s, 1H), 5.01 (s, 2H), 3.18 (s, 4H), 2.67 (bs, 2H), 2.34 (bs, 2H), 1.68 (bs, 2H), 1.54 (bs, 2H), 1.42 (s, 9H), 1.19 (bs, 16H), 0.81 (t, 3H, \(J = 4.5\) Hz); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 183.1, 166.7, 165.6, 149.6, 148.9, 123.3, 123.0, 109.6, 53.6, 53.0, 52.7, 39.9, 29.6, 31.9, 29.6, 29.5, 29.3, 28.9, 28.6, 28.0, 26.9, 25.4, 24.5, 22.7, 14.2; HRMS (ESI) calcd for C\(_{27}\)H\(_{47}\)N\(_7\)O\(_3\) (M\(^+\)) 517.3740, found 517.3736. Following the general deprotection procedure, **2.34a** (0.113 g, 0.22 mmol) afforded 0.072 g (80%) of the title compound as a
yellow foam: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.50 (bs, 1H), 6.54 (bs, 1H), 5.4 (s, 2H), 3.21 (bs, 2H), 2.51 (bs, 2H), 1.84 (bs, 4H), 1.51 (bs, 4H), 1.26 (s, 16H), 0.86 (bs, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 176.5, 165.8, 156.5, 128.7, 109.9, 54.9, 40.7, 37.8, 32.9, 30.5, 30.3, 30.1, 29.1, 28.8, 28.4, 27.9, 25.5, 25.3, 24.8, 23.6, 14.4; HRMS (ESI) calcd for C$_{22}$H$_{39}$N$_7$O (M$^+$) 417.3216, found 417.3211.

2.35b

2-{4-[5-(2-amino-1$H$-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl}-N-phenethyl-acetamide hydrochloride (2.35b): Following the general Click reaction, 2-amino-4-hept-6-ynyl-imidazole-1-carboxylic acid tert-buty l ester, 2.33 (0.075g, 0.270 mmol) was reacted with 2-azido-N-phenethyl-acetamide, 2.14 (0.054g, 0.270 mmol) affording 2.35a: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.43 (s, 1H), 7.20 (m, 5H), 6.83 (bs, 1H), 6.49 (s, 1H), 4.99 (s, 2H), 3.47 (s, 2H), 2.77 (t, 2H, $J = 6.8$ Hz), 2.70 (bs, 2H), 2.36 (bs, 2H), 1.68 (bs, 4H), 1.58 (s, 9H), 1.47 (bs, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 181.9, 165.7, 150.3, 148.9, 138.5, 128.8, 128.7, 126.7, 122.6, 106.5, 85.9, 53.0, 41.1, 35.4, 29.1, 28.8, 28.1, 27.6, 25.5; HRMS (ESI) calcd for C$_{25}$H$_{33}$N$_7$O$_3$ (M$^+$) 481.2801, found 481.2799. Following the general deprotection procedure, 2.35a (0.076 g, 0.16 mmol) afforded 0.060 g (74%) of the title compound as a yellow foam: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.23 (s, 2H), 7.23-7.19 (m, 5H), 6.51 (s, 1H),
5.32 (s, 2H), 3.48 (t, 1H, J = 7.6 Hz), 2.84 (m, 4H), 2.51 (t, 2H, J = 7.6 Hz), 1.77 (bs, 2H), 1.66 (t, 2H, J = 7.2 Hz), 1.45 (bs, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 167.8, 148.5, 147.0, 129.9, 129.7, 128.8, 128.3, 127.6, 109.8, 54.8, 42.4, 36.4, 29.3, 28.9, 25.3, 24.8; HRMS (ESI) calcd for C$_{20}$H$_{27}$N$_{7}$O (M$^+$) 381.2277, found 381.2275.

2.36b

2-\{4-\{5-\{2-amino-1H-imidazol-4-yl\}-pentyl\}\{-[1,2,3]triazol-1-yl\}\{-N-phenyl-acetamide\} hydrochloride (2.36b): Following the general Click procedure, 2-amino-4-hept-6-ynyl-imidazole-1-carboxylic acid tert-butyl ester, 2.33 (0.075g, 0.270 mmol) was reacted with 2-azido-N-phenyl-acetamide, 2.12 (0.072g, 0.410 mmol) affording 2.36a: $^1$H NMR (300 MHz, CDCl$_3$) δ 9.48 (s, 1H), 7.499 (m, 2H), 7.24 (m, 2H), 7.06 (t, 1H, J = 6.9 Hz), 6.48 (s, 1H), 5.21 (s, 2H), 2.67 (s, 2H), 2.32 (s, 2H), 1.62 (bs, 4H), 1.56 (s, 9H), 1.35 (bs, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 164.0 149.8, 148.8, 147.6, 137.5, 129.1, 128.7, 125.0, 123.1, 120.4, 109.8, 85.0, 53.5, 30.9, 29.1, 28.8, 28.1, 25.5; HRMS (ESI) calcd for C$_{23}$H$_{31}$N$_{7}$O$_3$ (M$^+$) 453.2488, found 453.2487. Following the general deprotection procedure, 2.36a (0.064 g, 0.14 mmol) afforded 0.050 g (51%) of the title compounds as a yellow foam: $^1$H NMR (400
MHz, CD$_3$OD) $\delta$ 8.53 (s, 1H), 7.59 (d, 2H, $J = 7.6$ Hz), 7.31 (t, 2H, $J = 7.2$ Hz), 7.11 (t, 1H, $J = 7.2$ Hz), 6.50 (s, 1H), 5.62 (s, 2H), 2.90 (bs, 2H), 2.50 (t, 2H, $J = 6.8$ Hz), 1.79 (bs, 2H), 1.66 (bs, 2H), 1.45 (bs, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 164.3, 148.5, 139.2, 130.1, 128.9, 125.9, 121.2, 109.8, 55.7, 29.3, 29.1, 28.9, 25.3, 24.7; HRMS (ESI) calcd for C$_{18}$H$_{24}$N$_{7}$O ($M^+$) 353.1964, found 353.1968.

(2.37)

(E)-2-methyl-3-phenylprop-2-en-1-amine (2.37): Triphenyl phosphine (0.551 g, 2.10 mmol) was dissolved in THF (3 mL). To a solution of (E)-(3-azido-2-methylprop-1-enyl)benzene (0.364 g, 2.1 mmol) in THF (2 mL) the dissolved PPh$_3$ was added dropwise and stirred for 16 h. H$_2$O (0.57 mL, 3.2 mmol) was added to the reaction and stirred for an additional 24 h. The reaction mixture was extracted with Et$_2$O (10 mL), dried (Na$_2$SO$_4$), filtered, and concentrated to dryness. The crude solid was purified via flash column chromatography (1-10% MeOH/DCM) and afforded (E)-2-methyl-3-phenylprop-2-en-1-amine, 2.37 (0.1678 g, 59%) as a clear oil: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26 (m, 5H), 6.41 (s, 1H), 3.34 (s, 2H), 1.186 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 139.5, 137.9, 128.5, 128.1, 126.1, 123.8, 50.3, 16.3; HRMS (ESI) calcd for C$_{10}$H$_{13}$N ($M^+$) 147.1048, found 147.1051.
(E)-2-azido-N-(2-methyl-3-phenylallyl)acetamide (2.38): Following the same procedure as 2-azido-N-decylacetamide, 2.4, 0.167 g (1.13 mmol) of (E)-2-methyl-3-phenylprop-2-en-1-amine, 2.38, afforded 0.250 g (98%) of the title compound as a tan solid: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.33 (m, 2H), 7.23 (m, 3H), 6.40 (s, 1H), 4.06 (s, 2H), 4.01 (d, 2H, $J = 5.7$ Hz), 1.87 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 166.7, 137.3, 134.3, 129.0, 128.4, 127.9, 126.9, 53.0, 47.3, 16.3; HRMS (ESI) calcd for C$_{12}$H$_{14}$N$_4$O (M$^+$) 230.11676, found 230.11662.

(E)-2-(4-(5-(2-amino-1H-imidazol-4-yl)pentyl)-1H-1,2,3-triazol-1-yl)-N-(2-methyl-3-phenylallyl)acetamide hydrochloride (2.39b): Following the general Click procedure, 2-amino-4-hept-6-ynyl-imidazole-1-carboxylic acid tert-butyl ester, 2.33 (0.075 g, 0.27 mmol) was reacted with (E)-2-azido-N-(2-methyl-3-phenylallyl)acetamide, 2.38, (0.186 g, 0.81 mmol) affording 2.39a: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.55 (s, 1H), 7.26 (m, 5H), 6.48 (s, 1H), 6.30 (s, 1H), 5.11 (s, 2H), 3.94 (s, 2H), 2.70 (s, 2H), 2.33 (s, 2H), 1.79 (s, 3H), 1.57 (s, 9H), 1.37 (bs, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 165.8, 149.7, 148.8, 148.1, 137.3, 134.0, 128.9, 128.2, 126.7, 126.5, 122.8, 106.7, 85.0, 53.0, 47.5, 29.0, 28.7, 28.1, 25.4, 16.2; HRMS
(ESI) calcd for $\text{C}_{27}\text{H}_{37}\text{N}_{7}\text{O}_{3} (\text{M}^+) 507.2958$, found 507.2959. Following the general deprotection procedure, 2.39a (0.058 g, 0.12 mmol) afforded 0.047 g (47%) of the title compound as a yellow foam: $^1\text{H NMR (400 MHz, CD}_3\text{OD)} \delta 8.49 (\text{s, } 1\text{H}), 7.25 (\text{m, } 5\text{H}), 6.49 (\text{s, } 1\text{H}), 6.46 (\text{s, } 1\text{H}), 5.48 (\text{s, } 2\text{H}), 3.97 (\text{s, } 2\text{H}), 2.87 (\text{bs, } 2\text{H}), 2.52 (\text{d, } 2\text{H, } J = 6.8 \text{ Hz}), 1.86 (\text{s, } 3\text{H}), 1.79 (\text{bs, } 2\text{H}), 1.66 (\text{bs, } 2\text{H}), 1.45 (\text{bs, } 2\text{H}); ^{13}\text{C NMR (100 MHz, CD}_3\text{OD)} \delta 166.5, 158.5, 148.5, 138.9, 135.4, 130.0, 129.3, 128.9, 127.6, 109.8, 95.8, 55.1, 29.3, 29.2, 28.9, 25.3, 24.8, 16.5; \text{HRMS (ESI) calcd for C}_{22}\text{H}_{29}\text{N}_{7}\text{O} (\text{M}^+) 407.2434$, found 407.2432.

2-azido-$\text{N}$-octylacetamide (2.40): Following the same procedure for 2-azido-$\text{N}$-decylacetamide, 2.3, 1.00 g (7.7 mmol) of octylamine afforded 1.35 g (96%) of the title compound as a brown oil: $^1\text{H NMR (300 MHz, CDCl}_3) \delta 6.43 (\text{s, } 1\text{H}), 3.92 (\text{s, } 2\text{H}), 3.23 (\text{q, } 2\text{H, } J = 6.9, 6.3 \text{ Hz}) 1.48 (\text{m, } 2\text{H}), 1.24 (\text{s, } 12\text{H}), 0.84 (\text{t, } 3\text{H, } J = 6.6 \text{ Hz}); ^{13}\text{C NMR (75 MHz, CDCl}_3) \delta 202.2, 52.8, 39.6, 31.8, 29.5, 29.3, 29.2, 26.9, 22.7, 14.1; \text{HRMS (ESI) calcd for C}_{10}\text{H}_{20}\text{N}_4\text{O} (\text{M}^+) 213.1710$, found 213.1711.

2-(4-(5-(2-amino-$1\text{H}$-imidazol-$4\text{-yl})$\text{p}$pentyl)-$1\text{H}$-$1,2,3$-triazol-$1\text{-yl}$-)N-octylacetamide hydrochloride (2.41b): Following the general Click procedure, 2-amino-$4$-hept-$6$-ynyl-
imidazole-1-carboxylic acid tert-butyl ester, \( \text{2.33} \) (0.075 g, 0.27 mmol) was reacted with 2-azido-N-octylacetamide, \( \text{2.40} \) (0.224 g, 0.81 mmol) affording tert-butyl 2-amino-4-(5-(1-(2-(octylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)pentyl)-1H-imidazole-1-carboxylate, \( \text{2.41a} \):

\[
\begin{align*}
\text{H NMR (300 MHz, CDCl}_3 \text{)} & \delta 7.51 (s, 1H), 6.56 (s, 1H), 5.02 (s, 2H), 3.23 (bs, 2H), 2.79 (bs, 2H), 2.52 (bs, 2H), 1.60 (s, 9H), 1.45 (m, 6H), 1.23 (bs, 14H), 0.86 (t, 3H, \text{J} = 6.0 \text{ Hz}); \\
\text{C NMR (100 MHz, CDCl}_3 \text{)} & \delta 165.3, 150.2, 149.1, 148.6, 137.9, 122.4, 106.2, 84.8, 52.8, 39.7, 31.6, 29.6, 29.1, 29.0, 28.9, 28.6, 28.0, 27.8, 27.5, 26.7, 25.3, 22.5, 14.0; \\
\text{HRMS (ESI)} & \text{calcd for C}_{25}\text{H}_{43}\text{N}_7\text{O}_3 \text{(M}^+ \text{) 490.3500, found 490.3505. Following the general deprotection procedure, tert-butyl 2-amino-4-(5-(1-(2-(octylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)pentyl)-1H-imidazole-1-carboxylate, \( \text{2.41a} \), (0.011 g, 0.024 mmol) afforded 0.010 g (23%) of the title compound as a yellow foam: } \\
\text{H NMR (400 MHz, CD}_3\text{OD) } & \delta 8.44 (s, 1H), 6.51 (s, 1H), 5.40 (s, 2H), 3.24 (t, 2H, \text{J} = 7.2 \text{ Hz}), 2.90 (bs, 2H), 2.52 (t, 2H, \text{J} = 7.2 \text{ Hz}), 1.80 (bs, 2H), 1.68 (bs, 2H), 1.55 (bs, 2H), 1.49 (bs, 2H), 1.32 (bs, 10H), 0.89 (t, 3H, \text{J} = 6.0 \text{ Hz}); \\
\text{C NMR (100 MHz, CD}_3\text{OD) } & \delta 165.9, 148.6, 129.3, 128.9, 109.9, 55.3, 41.0, 33.1, 30.5, 30.4, 29.3, 29.1, 28.9, 28.1, 25.4, 24.5, 23.8, 14.6; \text{HRMS (ESI) calcd for C}_{20}\text{H}_{35}\text{N}_7\text{O} \text{(M}^+ \text{) 390.2976, found 390.2977.}
\end{align*}
\]

**Biological Screening Experimental**

**Procedure to Determine the Inhibition Effect of Test Compounds on A. baumannii and E. coli Biofilms:** Inhibition assays were performed by taking an overnight culture and subculturing it with an OD\textsubscript{600} of 0.01 into the necessary media; Luria-Mertanit (LB) for A.
baumannii and E. coli. Stock solutions of predetermined concentrations of the test compounds were prepared with DMSO (biology grade). These stock solutions were aliquoted (100 µL) into the wells of a 96-well PVC microtiter plate. Sample plates were wrapped in GLAD Press n’ Seal® followed by incubation under stationary conditions for 24 h at 37 °C. After incubation the media was discarded and the plates were washed with water. The sample plates were stained with 110 µL of 0.1% solution of crystal violet (CV) and incubated at ambient temperature for 30 min. The CV stain was discarded and the plates were washed with water. The remaining stain was solubilized with 200 µL of 95% ethanol. After the biofilms was dissolved (5 minutes) a sample of 125 µL of solublized CV stained ethanol was transferred from each well into the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD$_{540}$ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out. The percent inhibition was calculated by the comparison of the OD$_{540}$ for established biofilm (control) versus treated established biofilm (compound treated) under identical conditions.

Procedure to Determine the Dispersal Effect of Test Compounds on E. coli Preformed Biofilms: An overnight culture of E. coli bacteria was subcultured at an OD$_{600}$ of 0.05 into LB media and then aliquoted (100 µL) into a 96 well plate. The plate was covered with GLAD Press n’ Seal® and incubated at 37 °C for 24 hours, after which the media was discarded and the plate was washed with water. Then fresh media with the appropriate concentration of compound was added to the wells. The plate was then covered again in the
same manner, incubated at 37 °C for 24 hours, the media was discarded and the plate was washed with water. The wells were then incubated with 0.1% CV (110 µL) and allowed to stand at ambient temperature for 30 minutes. After the CV was discarded and the plate was thoroughly washed with water. Then the remaining stain was solubilized with 200 µL of 95% ethanol and allowed to stand for 5 minutes. Biofilm dispersion was quantified by measuring the OD$_{540}$ for each well by transferring 125 µL of CV stained ethanol to a 96 well reader plate. The percent dispersion was calculated the same way as for inhibition.

**Growth Curve Analysis (To determine the toxicity of the test compound at its corresponding IC$_{50}$ value):** In the growth curve assay, bacterial cell density was evaluated in the appropriate media by taking its optical density (OD$_{540}$). The bacteria were cultured overnight and the OD$_{600}$ of 0.01 was taken and used to determine the optical density of the bacteria. In three test tubes the control was prepared in one tube with the appropriate media and bacteria and in the other two test tubes the IC$_{50}$ concentration of compound, media and bacteria were combined. The three test tubes were placed at 37 °C and the OD$_{600}$ was determined at time (t) = 1, 3, 5, 7, 24 hours. A compound was determined to be non-toxic if comparable ODs were observed at the same time points. Toxicity can also be determined qualitatively by determining the degree of cloudiness of each test tube at each time point, clear indicates that the compounds are killing the bacteria.

**Red Blood Cell Hemolysis Assay:** Hemolysis assays were performed on mechanically defibrinated sheep’s blood (DSB100, Hemostat Labs, Dixon, USA). A sample (1.5 mL) of
blood was placed into a microcentrifuge tube and separated at 10000 rpm for 10 min. The supernatant was removed, and the cells were resuspended in phosphine-buffered saline (PBS; 1mL). The suspension was centrifuged, the supernatant was removed, and the cells were resuspended two more times. The final cell suspension was then diluted tenfold. Test compound solutions were made in PBS in small culture tubes and then added to aliquots of the tenfold-diluted suspension. PBS alone was used as a negative control and as a zero hemolysis marker, while a 1% Triton X sample was used as a positive control and as the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm. After 1 h, the samples were transferred to microcentrifuge tubes and then separated at 1000- rpm for 10 min. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was measured on a UV spectrometer at a 540 nm wavelength.15

REFERENCES


APPENDICES
NMR Spectra
2.26a
**NMR Data**

**Signal Position:**
- **Solvent:** CDCl3
- **Temperature:** Ambient
- **Compounds:** Investigating "Acetic Acid"

**Details:**
- **Volume:** 1.0 mmol
- **Pulse:** 5.0° Degree
- **Delay:** 360 sec
- **Spectrometer:** Bruker AV-400D 1H 19F/13C
- **Field:** 9.41 T
- **Scan:** 32768
- **Resolution:** 30 Hz
- **Total Time:** 3 min, 3 sec

**Chemical Shifts:**
- 6.205, 7.558, 7.907
- 4.235, 7.90
- 2.80, 2.84, 3.20
- 1.80, 1.40, 0.86
- 0.40, 0.20, 0.80

**2.35a**
Inhibition Dose Response Curves

E. coli: K-12 ER2738
Dispersion Dose Response Curves

E. coli K-12 ER2738

% Inhibition vs. Concentration (µM)

% Dispersion vs. Concentration (µM)
Growth Curve Analysis

**E. coli K-12 ER2738**

- **Optical Density (600 nm)**
  - Blue diamond: "2.19b (40 uM)"
  - Pink square: Control

**Chemical Structure**: ![Chemical Structure](image)

**Time (hours)**

0 5 10 15 20 25 30

0 0.5 1 1.5 2 2.5 3 3.5
Red Blood Cell Hemolysis Assay

Catheter Study
CHAPTER 3
A NEW CLASS OF 2-AI CONJUGATES INSPIRED BY A
2-AMINOBENZIMIDAZOLE

3.1 2-Aminobenzimidazole Study

Bromoageliferin and oroidin are two alkaloids that have been reported to have both antibiotic and biofilm inhibition activities. Our group has shown through our synthetic analogues of these natural products that they can be either toxic or non-toxic depending on the concentration of the compound, the bacterial species, and the architecture of the compounds. Previously, a library of 55 2-aminobenzimidazoles (2-ABI) were synthesized and screened for their antibacterial activity. The 2-ABIs are considered to be a simplified version of bromoageliferin and recently benzimidazole derivatives have received attention for their biological activity.

The compounds of this library were screened for their antibiotic activity by determining its minimum inhibitory concentration (MIC) values using standard dilution protocols. MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of a microorganism. The compounds were screened in a 96-well microtiter plate starting at 400 µM and then making 10 two-fold serial dilutions down to 391 nM. The plates were inspected for visible growth after 16 hours of incubation at 37 °C. The compounds were screened against MRSA (BAA-44), three other strains of S. aureus (ATCC-29213, ATCC-29740, and ATCC-25923), A. baumannii (ATCC-19606), and a MDR strain of A. baumannii (MDRAB BAA-1605), and three clinical isolates (AB0043, UH8407, and 3340). The library had some compounds that showed no antimicrobial activity at the highest
concentration (400 µM) and some that had activity between 100 µM and 6.25 µM. Compound 3.1 had the best activity against all of the strains tested (Table 3.1) making it the lead compound of the library.[1]

**Figure 3.1:** Lead compound 3.1 from 2-ABI library.

In this study, compound 3.1 was chosen as the lead structure for further SAR studies to enhance antibiotic activity.

**Table 3.1:** MIC values of 3.1 against *S. aureus* and *A. baumannii* strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>S. aureus</em> Strains</th>
<th><em>A. baumannii</em> Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA (BAA-44)</td>
<td>ATCC-29213</td>
</tr>
<tr>
<td></td>
<td>ATCC-29740</td>
<td>ATCC-25923</td>
</tr>
<tr>
<td></td>
<td>ATCC-19606</td>
<td>MDRAB (BAA-1605)</td>
</tr>
<tr>
<td></td>
<td>ABOO43</td>
<td>3340</td>
</tr>
<tr>
<td>3.1</td>
<td>12.5 µM</td>
<td>12.5 µM</td>
</tr>
<tr>
<td></td>
<td>6.25 µM</td>
<td>12.5 µM</td>
</tr>
<tr>
<td></td>
<td>25 µM</td>
<td>12.5 µM</td>
</tr>
<tr>
<td></td>
<td>12.5 µM</td>
<td>12.5 µM</td>
</tr>
<tr>
<td></td>
<td>25 µM</td>
<td></td>
</tr>
</tbody>
</table>

Control compounds were synthesized to test the necessity of the 2-ABI head unit, and one in particular was the removal of the aniline amide moiety (Figure 3.2).

**Figure 3.2:** Removal of the aniline amide moiety to generate a new 2-AI library.
The new compound from the SAR study was screened for antibiotic activity against MRSA. The control 2-AI compound demonstrated the same activity as compound 3.0 against MRSA with an MIC value of 12.5 µM. This result concluded that the 2-AI was necessary for antibiotic activity, either as just the 2-AI or as the 2-ABI and was the inspiration for the next synthetic library.

3.2 A New Class of 2-AI Analogues

Removing the aniline amide moiety of the lead 2-ABI had no affect on the antibiotic activity against MRSA. The activity of the 2-AI derivative provided an opportunity to synthesize a new library and test for both antibiotic and anti-biofilm activity.

First we synthesized 2-AI conjugates that varied from the lead by aliphatic chain length, from butyl to nonyl. The synthesis of the 2-AI library was rapidly completed in a total of four steps. The commercially available benzoyl chlorides (n = 1-6) were subjected to homologation via a reaction with diazomethane, and then quenched with hydrobromic acid to afford the α-bromoketone. The various α-bromoketones were cyclized with Boc-guanidine and then, after purification, were deprotected to obtain the desired product (Scheme 3.1).
Next, we took advantage of the vast commercially available benzoyl chlorides and investigated different substituents on the aryl ring. These benzoyl chlorides were subjected to the same 4 step synthetic pathway as seen in Scheme 3.2 to afford the new 2-AI conjugates.

**Scheme 3.1**: Synthesis of 2-AI, 3.2-3.6c from commercially available benzoyl chlorides.

**Scheme 3.2**: Synthesis of 2-AI 3.7-3.11c with various aryl substituents.
These commercially available benzoyl chlorides were transformed into a total of 10 new 2-Al conjugates (Figure 3.3) to be screened for antibiotic and antibiofilm activity.

![Chemical structures](image)

**Figure 3.3**: New 2-Al conjugates.

### 3.3 Biological Studies of 2-Al Analogues

First, the compounds were screened for anti-MRSA activity since compound 3.2c already demonstrated that it was active against MRSA with an MIC value of 12.5 µM. The anti-MRSA results showed that as the hydrocarbon tail length increases from 4 to 5, activity increases by two-fold, from 5 to 7 activity remains constant, and from 7 to 9 activity increases again by two-fold (Table 3.2). The biphenyl 2-Al conjugate had the best activity for the non-linear chain lengths with an MIC of 12.5 µM.
Table 3.2: MIC values for compounds 3.2-3.11c against MRSA.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>25 µM</td>
</tr>
<tr>
<td>3.2c</td>
<td>12.5 µM</td>
</tr>
<tr>
<td>3.4c</td>
<td>12.5 µM</td>
</tr>
<tr>
<td>3.5c</td>
<td>12.5 µM</td>
</tr>
<tr>
<td>3.6c</td>
<td>6.25 µM</td>
</tr>
<tr>
<td>3.7c</td>
<td>&gt;400 µM</td>
</tr>
<tr>
<td>3.8c</td>
<td>50 µM</td>
</tr>
<tr>
<td>3.9c</td>
<td>&gt;400 µM</td>
</tr>
<tr>
<td>3.10c</td>
<td>12.5 µM</td>
</tr>
<tr>
<td>3.11c</td>
<td>50 µM</td>
</tr>
</tbody>
</table>

Next we wanted to see how the MIC activity would be when they were subjected to Gram-negative bacteria, such as *A. baumannii* and *E. coli*. This time the trends were not the same. For example, against *A. baumannii* the pentyl (3.2c) and hexyl (3.4c) linear aliphatic chains had the best activity, with activity dropping two-fold when it is a butyl and heptyl chain, while the nonyl aliphatic chain (3.6c) displayed an MIC of >400 µM. The biphenyl, derivative 3.10c, had the best activity against the *A. baumannii* for the aryl substituents with an MIC of 25 µM. The phenyl did not have activity again with an MIC value of >400 µM. When the compounds were tested against one other Gram-negative strain, *E. coli*, they showed about the same activity (Table 3.3). The most active was compound 3.4c with an MIC of 12.5 µM.
Table 3.3: MIC values for 3.2-3.11c against A. baumannii and E. coli.

<table>
<thead>
<tr>
<th></th>
<th>A. baumannii</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>50 µM</td>
<td>50 µM</td>
</tr>
<tr>
<td>3.2c</td>
<td>12.5 µM</td>
<td>25 µM</td>
</tr>
<tr>
<td>3.4c</td>
<td>12.5 µM</td>
<td>12.5 µM</td>
</tr>
<tr>
<td>3.5c</td>
<td>50 µM</td>
<td>25 µM</td>
</tr>
<tr>
<td>3.6c</td>
<td>&gt;400 µM</td>
<td>&gt;400 µM</td>
</tr>
<tr>
<td>3.7c</td>
<td>&gt;400 µM</td>
<td>&gt;400 µM</td>
</tr>
<tr>
<td>3.8c</td>
<td>100 µM</td>
<td>100 µM</td>
</tr>
<tr>
<td>3.9c</td>
<td>&gt;400 µM</td>
<td>&gt;400 µM</td>
</tr>
<tr>
<td>3.10c</td>
<td>25 µM</td>
<td>25 µM</td>
</tr>
<tr>
<td>3.11c</td>
<td>200 µM</td>
<td>200 µM</td>
</tr>
</tbody>
</table>

Following the antibiotic study we wanted to investigate if the 2-AI conjugates had any anti-biofilm activity since most of our libraries based on a 2-AI scaffold modulate biofilms. The 2-ABI conjugates in the previous library were not able to inhibit or disperse biofilms. The 2-AI compounds were initially screened for inhibition at 100 µM against both A. baumannii and E. coli. The results for the inhibition screens against both A. baumannii and E. coli are summarized in Table 3.4.

Table 3.4: Initial biofilm inhibition results.

<table>
<thead>
<tr>
<th></th>
<th>% Inhibition of A. baumannii Biofilms (at 100 µM)</th>
<th>% Inhibition of E. coli Biofilms (at 100 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>3.2c</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>3.4c</td>
<td>99%</td>
<td>95%</td>
</tr>
<tr>
<td>3.5c</td>
<td>99%</td>
<td>94%</td>
</tr>
<tr>
<td>3.6c</td>
<td>99%</td>
<td>92%</td>
</tr>
<tr>
<td>3.7c</td>
<td>22.5%</td>
<td>-43.2%</td>
</tr>
<tr>
<td>3.8c</td>
<td>39.6%</td>
<td>93.8%</td>
</tr>
<tr>
<td>3.9c</td>
<td>27.4%</td>
<td>0.4%</td>
</tr>
<tr>
<td>3.10c</td>
<td>97%</td>
<td>97.6%</td>
</tr>
<tr>
<td>3.11c</td>
<td>40.2%</td>
<td>64.1%</td>
</tr>
<tr>
<td>2-ABI lead (3.1)</td>
<td>66%</td>
<td>51.2%</td>
</tr>
</tbody>
</table>
Compounds that had greater than 80% inhibition against the bacteria were screened for inhibition at a lower concentration of 50 µM. These results can be seen in Table 3.5.

Table 3.5: % Inhibition of biofilms at 50 µM.

<table>
<thead>
<tr>
<th></th>
<th>A. baumannii</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>93%</td>
<td>99.1%</td>
</tr>
<tr>
<td>3.2c</td>
<td>98%</td>
<td>98.9%</td>
</tr>
<tr>
<td>3.4c</td>
<td>98%</td>
<td>97.7%</td>
</tr>
<tr>
<td>3.5c</td>
<td>97%</td>
<td>99.3%</td>
</tr>
<tr>
<td>3.6c</td>
<td>35.4%</td>
<td>83.3%</td>
</tr>
<tr>
<td>3.8c</td>
<td>-</td>
<td>53%</td>
</tr>
<tr>
<td>3.10c</td>
<td>95%</td>
<td>98%</td>
</tr>
</tbody>
</table>

The results show that by removal of the aniline amide moiety the compounds are able to inhibit biofilms in addition to their antibiotic activity. Compound 3.10c displayed similar activity to compounds 3.2 – 3.5c as biofilm inhibitors against A. baumannii and E. coli. On the other hand, 3.6c, 3.7c, 3.8c, and 3.11c were not strong biofilm inhibitors against A. baumannii and 3.7c promoted biofilm formation against E. coli, and 3.7c did not inhibit the E. coli biofilms. However, 3.8c had inhibition activity against E. coli biofilms by >90% at 100 µM.

The compounds were then screened against A. baumannii and E. coli for dispersion activity. Initially compounds 3.2-3.11c were screened against E. coli at 400 µM and any compound that had >90% dispersion was carried on for further screening. A dose response curve for compounds 3.2c, 3.3c, and 3.10c was completed in order to determine the compounds respective EC$_{50}$ values (Table 3.6).
Table 3.6: EC<sub>50</sub> values for *E. coli*.

<table>
<thead>
<tr>
<th>EC&lt;sub&gt;50&lt;/sub&gt; Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>39.0 ± 1.9 µM</td>
</tr>
<tr>
<td>3.2c</td>
<td>26.8 ± 0.94 µM</td>
</tr>
<tr>
<td>3.10c</td>
<td>47.5 ± 11.2 µM</td>
</tr>
</tbody>
</table>

The compounds were then screened for dispersal activity against *A. baumannii* at 100 µM. None of the compounds dispersed >90% at 100 µM, so the compounds that had >40% at 100 µM had a dose response curve performed to determine their EC<sub>50</sub> values against *A. baumannii*. Compounds 3.2c, 3.3c, and 3.4c had the best dispersal activity against *A. baumannii* and their EC<sub>50</sub> values are displayed in Table 3.7.

Table 3.7: EC<sub>50</sub> values for *A. baumannii*.

<table>
<thead>
<tr>
<th>EC&lt;sub&gt;50&lt;/sub&gt; Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3c</td>
<td>173.3 ± 28.0 µM</td>
</tr>
<tr>
<td>3.2c</td>
<td>151.0 ± 7.6 µM</td>
</tr>
<tr>
<td>3.4c</td>
<td>218.6 ± 32.7 µM</td>
</tr>
</tbody>
</table>

Given that the 2-ABI conjugates were not able to disperse biofilms, and 3.2c had the most potent EC<sub>50</sub> values, 26.8 µM and 151.0 µM, against *E. coli* and *A. baumannii*, respectively, 3.2c was deemed the most active compound in this study. Compound 3.2c also displayed some of the best MIC values against MRSA, *A. baumannii*, and *E. coli*, as well as it was able to inhibit biofilm formation for both *E. coli* and *A. baumannii* >90% at 50 µM.

3.4 SAR/Control Study of Lead 2-AI Analogue

With 3.2c being the lead 2-AI analogue for the study, an SAR study was completed to discover which components of the compound were essential for inhibition, dispersion, and
antibiotic activity. A total of 12 variations, including amide and ester conjugates of 3.2c were synthesized and screened for activity.

First, removing the 2-Al head component of 3.2c and introducing various amides and esters onto the tail of 3.2c were investigated. Pentyl benzoyl chloride was used as the synthetic building block for the synthesis of the amides and esters (Scheme 3.3) from commercially available amines and alcohols.

Scheme 3.3: Synthesis of amides (3.12-3.17) and esters (3.18-3.21).
Next in the SAR study, instead of completely removing the 2-AI head group completely, alterations to the head group were investigated. For example, instead of cyclizing with Boc-guanidine to arrive at the Boc-protected 2-AI, the α-bromoketone was cyclized with thiourea to arrive at the 2-aminothiazole (Scheme 3.4).[3]

Scheme 3.4: Synthesis of 2-aminothiazole 3.22.
In addition to synthesizing 3.22, pentyl benzoyl chloride was reacted with commercially available bromo-ethylamine to afford the dihydro-oxazole derivative, 3.23 (Scheme 3.5).[^4]

![Scheme 3.5](image)

**Scheme 3.5**: Synthesis of dihydro-oxazole derivative, 3.23.

These compounds 3.12-3.21, 3.22 and 3.23 were subjected to an initial screen to determine their MIC against MRSA, and all of them reported an MIC >400 µM. With this result, the compounds were not carried on for further MIC screens. Compounds 3.12-3.21 were screened for biofilm inhibition at 400 µM against *A. baumannii* and did not modulate the biofilms. On the other hand, 3.22 and 3.23 were screened against both *A. baumannii* and *E. coli* at 100 µM with 28.6% and 28.3% inhibition against *A. baumannii* and promoted *E. coli* biofilm growth by 5% and 46% respectively. Given the fact that they were able to modulate *A. baumannii* biofilms at 100 µM, 3.22 and 3.23 were also subjected to dispersion screens against *A. baumannii* at 100 µM, resulting in 21% and 18% dispersion, respectively.

**3.5 Conclusion**

In conclusion, a library of 2-AI analogues based off of a previously synthesized 2-ABI scaffold was synthesized and screened for biological activity. The 2-AI analogues were able to modulate both *A. baumannii* and *E. coli* biofilms in comparison to the lack of biofilm activity for the 2-ABI compounds. Dose response experiments were performed on the active 2-AI analogues that had decent dispersal activity against both *A. baumannii* and *E. coli*. 3.2c,
3.3c, and 3.10c were the most active compounds against *E. coli* with EC\(_{50}\) values of 39.0 ± 1.9 µM, 26.8 ± 0.94 µM, and 47.5 ± 11.2 µM respectively. 3.2c, 3.3c, and 3.4c exhibited the best dispersal activity against *A. baumannii* biofilms with EC\(_{50}\) values of 173.3 ± 28.0 µM, 151.0 ± 7.6 µM, and 218.6 ± 32.7 µM. The 2-AI analogues were subjected to susceptibility screens and displayed antibiotic behavior against *A. baumannii, E. coli*, and MRSA. The results illustrate that the 2-AI analogues act as antibiotics that also have the ability to disperse, which is important for modulation of biofilms. In conclusion, 3.2c was determined to be the lead compound of the study, and an SAR study on the structures was performed. The control biological results from the SAR study concluded that the 2-AI head was essential for modulation of biofilms as well as antibiotic behavior.

### 3.6 Experimental

All reagents used for chemical synthesis were purchased commercially available sources and used without further purification. Chromatography was performed using 60 mesh standard silica gel from Sorbtech. NMR solvents were obtained from Cambridge Isotope Labs and were used as is. \(^1\)H NMR (300 MHz or 400 MHz) and \(^13\)C NMR (75 MHz or 100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvents; coupling constants (\(J\)) are in hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, bt = broad triplet, q = quartet, m = multiplet, bm = broad multiplet. Mass spectra were obtained at the NCSU
Department of Chemistry Mass Spectrometry Facility. Funding was obtained from NCSU Department of Chemistry.

2-bromo-1-(4-butylphenyl)ethanone (3.3a): 4-butyl benzoyl chloride (2.2 g, 11.0 mmol) was added drop-wise to a 0 °C solution of CH$_2$N$_2$ (33.0 mmol generated from Diazald®/KOH) in Et$_2$O (100 mL). This solution was stirred at 0 °C for 1 h upon which the reaction was quenched via the drop-wise addition of 48% solution of conc. HBr (4.0 mL). The reaction mixture was diluted with dichloromethane (15 mL) and immediately washed with sat. NaHCO$_3$ (3 x 25 mL) and brine (2 x 25 mL) before being dried (Na$_2$SO$_4$) filtered and concentrated. The crude oil was purified via flash column chromatography (100% DCM) to obtain 2-bromo-1-(4-butylphenyl)ethanone (2.76 g, 98%) as a pale yellow oil: $^1$H NMR (400 MHz, DMSO) δ 7.94 (d, 2H, $J = 8.0$ Hz), 7.36 (d, 2H, $J = 8.0$ Hz), 4.89 (s, 2H), 2.65 (t, 2H, $J = 7.6$ Hz), 1.56 (quintet, 2H, $J = 8.0$ Hz), 1.29 (sextet, 2H, $J = 7.6$ Hz), 0.89 (t, 3H, $J = 7.6$ Hz); $^{13}$C NMR (100 MHz, DMSO) δ 191.1, 149.0, 131.7, 128.9, 128.7, 128.5, 34.8, 33.9, 32.7, 21.7, 13.7; HRMS (ESI) calcd for C$_{12}$H$_{15}$BrO (M$^+$) 255.0379, found 255.0384.
2-bromo-1-(4-hexylphenyl)ethanone (3.4a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 4-hexyl benzoyl chloride (2.47 g, 11.0 mmol) afforded 3.09 g (99%) of the title compound as a pale yellow oil: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.90 (d, 2H, $J = 6.6$ Hz), 7.28 (d, 2H, $J = 7.8$ Hz), 4.43 (s, 2H), 2.67 (t, 2H, $J = 7.8$ Hz), 1.64 (m, 2H), 1.27 (s, 6H), 0.88 (t, 3H, $J = 6.9$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 191.1, 150.1, 131.8, 129.3, 129.1, 36.2, 31.8, 31.2, 31.1, 29.1, 22.7, 14.2; HRMS (ESI) calcd for C$_{14}$H$_{19}$BrO (M$^+$) 283.0692, found 283.0694.

2-bromo-1-(4-heptylphenyl)ethanone (3.5a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 4-heptyl benzoyl chloride (2.62g, 11.0 mmol) afforded 3.21 g (98%) of the title compound as a yellow oil: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.84 (d, 2H, $J = 8.4$ Hz), 7.22 (d, 2H, $J = 7.8$ Hz), 4.38 (s, 2H), 2.60 (t, 2H, $J = 7.5$ Hz), 1.57 (bs, 2H), 1.21 (bs, 8H), 0.83 (t, 3H, $J = 4.8$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 190.7, 149.7, 131.6, 129.0, 128.8, 36.0, 31.7, 31.2, 31.0, 29.2, 29.1, 22.6, 14.1; HRMS (ESI) calcd for C$_{15}$H$_{21}$BrO (M$^+$) 297.0849, found 297.0852.
2-bromo-1-(4-nonylphenyl)ethanone (3.6a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 4-nonyl benzoylchloride (2.93 g, 11.0 mmol) afforded 3.13 g (88%) of the title compound as a white solid: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.9 (d, 2H, $J$ = 8.4 Hz), 7.27 (d, 2H, $J$ = 8.7 Hz), 4.42 (s, 2H), 2.65 (t, 2H, $J$ = 7.5 Hz), 1.60 (bs, 2H), 1.29 (bs, 12H), 0.87 (t, 3H, $J$ = 6.3 Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 191.0, 150.1, 131.7, 129.2, 129.1, 128.8, 128.5, 36.2, 32.0, 31.2, 31.1, 29.6, 29.5, 29.4, 29.3, 22.8, 14.2; HRMS (ESI) calcd for C$_{17}$H$_{25}$BrO (M$^+$) 325.1162, found 325.1158.

2-bromo-1-phenylethanone (3.7a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), benzoylchloride (0.773 g, 5.5 mmol) afforded 1.10 g (100%) of the title compound as a colorless oil: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.98 (d, 2H, $J$ = 7.5 Hz), 7.60 (d, 1H, $J$ = 6.3 Hz), 7.50 (d, 2H, $J$ = 7.2 Hz), 4.46 (s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 182.8, 134.1, 129.1, 129.0, 31.1; HRMS (ESI) calcd for C$_8$H$_7$BrO (M$^+$) 198.9753, found 198.9756.
2-bromo-1-(4-tert-butylphenyl)ethanone (3.8a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 4-t butyl benzoyl chloride (1.08 g, 5.50 mmol) afforded 1.30 g (93%) of the title compound as a yellow oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.92 (d, 2H, \(J = 8.4\) Hz), 7.49 (d, 2H, \(J = 8.4\) Hz), 4.34 (s, 2H), 1.34 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 191.0, 158.0, 129.1, 128.6, 125.9, 35.7, 31.2; HRMS (ESI) calcd for C\(_{12}\)H\(_{15}\)BrO (M\(^+\)) 255.0379, found 255.0378.

2-bromo-1-(2-nitrophenyl)ethanone (3.9a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 2-nitro benzoyl chloride (1.02 g, 5.50 mmol) afforded 1.34 g (100%) of the title compound as a brown oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.20 (d, 1H, \(J = 8.4\) Hz), 7.80 (m, 1H), 7.69 (m, 1H), 7.48 (d, 1H, \(J = 7.6\) Hz), 4.30 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 194.5, 135.0, 131.5, 129.2, 128.9, 124.6, 34.1; HRMS (ESI) calcd for C\(_8\)H\(_6\)BrNO\(_3\) (M\(^+\)) 243.9604, found 243.9602.
2-bromo-1-(biphenyl)ethanone (3.10a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), (1,1'-biphenyl)-4-carbonyl chloride (1.20 g, 5.5 mmol) afforded 1.10 g (73%) of the title compound as a yellow oil: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.06 (d, 2H, $J = 8.4$ Hz), 7.70 (d, 2H, $J = 8.4$ Hz), 7.63 (d, 2H, $J = 7.2$ Hz), 7.49 (t, 2H, $J = 7.2$ Hz), 7.43 (d, 1H, $J = 7.2$ Hz), 4.48 (s, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 191.1, 146.8, 139.7, 130.3, 129.7, 129.3, 129.2, 128.7, 127.6, 127.5, 127.2, 31.1; HRMS (ESI) calcd for C$_{14}$H$_{11}$BrO (M$^+$) 275.0066, found 275.0064.

2-bromo-1-(naphthalen-2-yl)ethanone (3.11a): In a similar manner as 2-bromo-1-(4-butylphenyl)ethanone (3.3a), 2-naphthoyl chloride (1.05 g, 5.5 mmol) afforded 0.986 g (70%) of the title compound as a tan solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.50 (s, 1H), 7.91 (m, 4H), 7.59 (m, 2H), 4.58 (s, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 191.1, 136.0, 132.5, 131.4, 131.1, 129.9, 129.2, 129.0, 127.2, 124.3, 31.2; HRMS (ESI) calcd for C$_{12}$H$_9$BrO (M$^+$) 248.9910, found 248.9907.
tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b): 2.76 g (10.8 mmol) of 2-bromo-1-(4-butylphenyl)ethanone (3.3a) was dissolved in 15 mL of anhydrous DMF and allowed to stir at ambient temperature. 5.15 g (32.4 mmol) of boc-guanidine was added to the round bottom flask and allowed to stir for 3 days. The reaction mixture was then concentrated to dryness and dissolved in 25 mL of EtOAc and immediately washed with H₂O (2 x 30 mL) and brine (30 mL) before being dried (Na₂SO₄) filtered and concentrated. The crude solid was then purified via flash column chromatography (10:1, 8:1, 6:1, 4:1 Hex:EtOAc) to obtain 0.598 g (18%) of the title compound as a tan solid: ¹H NMR (400 MHz, DMSO) δ 7.62 (d, 2H, J = 8.4 Hz), 7.15 (d, 2H, J = 8.8 Hz), 6.59 (s, 1H), 3.34 (s, 2H), 2.55 (m, 2H), 1.54 (s, 9H), 1.30 (m, 2H), 0.89 (t, 3H, J = 7.6 Hz); ¹³C NMR (100 MHz, DMSO) δ 150.7, 141.4, 137.4, 131.3, 128.7, 125.0, 105.9, 94.3, 84.9, 35.0, 33.5, 28.0, 22.2, 14.2; HRMS (ESI) calcd for C₁₈H₂₅N₃O₂ (M⁺) 316.202, found 316.2023.

tert-butyl 2-amino-4-(4-hexylphenyl)-1H-imidazole-1-carboxylate (3.4b): In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 3.09 g
(10.9 mmol) of 2-bromo-1-(4-hexylphenyl)ethanone (3.4a) afforded 1.60 g (43%) of the title compound as a tan solid: $^1$H NMR (400 MHz, DMSO) $\delta$ 7.62 (d, 2H, $J = 8.4$ Hz), 7.27 (s, 1H), 7.14 (d, 2H, $J = 8.0$ Hz), 6.59 (s, 1H), 2.54 (s, 2H), 1.57 (s, 9H), 1.40 (s, 4H), 1.27 (s, 4H), 0.84 (t, 3H, $J = 7.6$ Hz); $^{13}$C NMR (100 MHz, DMSO) $\delta$ 188.6, 151.2, 149.4, 141.5, 137.5, 131.3, 128.7, 125.0, 105.9, 85.0, 35.3, 31.5, 31.3, 28.8, 28.2, 27.9, 22.5, 14.3; HRMS (ESI) calcd for C$_{20}$H$_{29}$N$_3$O$_2$ (M$^+$) 344.2333, found 344.2337.

![Image](image.png)

**3.5b**

tert-butyl 2-amino-4-(4-heptylphenyl)-1H-imidazole-1-carboxylate (3.5b): In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 3.21 g (10.8 mmol) of 2-bromo-1-(4-heptylphenyl)ethanone (3.5a) afforded 1.73 g (44%) of the title compound as a tan solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.57 (d, 2H, $J = 7.6$ Hz), 7.15 (d, 2H, $J = 8.0$ Hz), 7.04 (s, 1H), 6.15 (s, 1H), 2.59 (t, 2H, $J = 7.6$ Hz), 1.61 (s, 9H), 1.45 (s, 4H), 1.27 (m, 6H), 0.87 (t, 3H, $J = 6.0$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 178.8, 151.4, 149.6, 142.3, 137.9, 130.7, 128.7, 125.1, 105.7, 85.2, 36.1, 31.6, 31.3, 29.5, 28.2, 28.1, 22.8, 14.2; HRMS (ESI) calcd for C$_{21}$H$_{31}$N$_3$O$_2$ (M$^+$) 358.2489, found 358.2495.
**tert-butyl 2-amino-4-(4-nonylphenyl)-1H-imidazole-1-carboxylate (3.6b):** In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 3.13 g (9.62 mmol) of 2-bromo-1-(4-nonylphenyl)ethanone (3.6a) afforded 1.14 g (31%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.58 (d, 2H, $J = 8.4$ Hz), 7.15 (d, 2H, $J = 8.4$ Hz), 7.04 (s, 1H), 6.21 (s, 1H), 2.59 (t, 2H, $J = 7.2$ Hz), 1.57 (s, 9H), 1.44 (s, 6H), 1.27 (m, 8H), 0.87 (t, 3H, $J = 6.8$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.9, 149.6, 142.3, 140.1, 136.9, 128.7, 125.1, 105.7, 85.1, 35.9, 32.1, 31.7, 29.8, 29.7, 29.5, 28.2, 28.1, 22.9, 14.3; HRMS (ESI) calcd for C$_{23}$H$_{35}$N$_3$O$_2$ (M$^+$) 386.2802, found 386.2807.

**tert-butyl 2-amino-4-phenyl-1H-imidazole-1-carboxylate (3.7b):** In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 0.40 g (2.0 mmol) of 2-bromo-1-phenylethanone (3.7a) afforded 0.23 g (45%) of the title compound a tan solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60 (d, 2H, $J = 8.4$ Hz), 7.26 (t, 2H, $J = 7.2$ Hz), 7.15 (d, 1H, $J = 8.4$ Hz), 7.01 (s, 1H), 6.34 (s, 1H), 1.52 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 151.1,
tert-butyl 2-amino-4-(4-tert-butylphenyl)-1H-imidazole-1-carboxylate (3.8b): In a similar manner as tert-butyl 2-amino-4-(4-butyphenyl)-1H-imidazole-1-carboxylate (3.3b), 0.400 g (1.6 mmol) of 2-bromo-1-(4-tert-butylphenyl)ethanone (3.8a) afforded 0.167 g (44%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.61 (d, 2H, $J = 8.8$ Hz), 7.37 (d, 2H, $J = 8.4$ Hz), 7.05 (s, 1H), 6.05 (s, 1H), 1.62 (s, 9H), 1.33 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.7, 150.4, 138.0, 130.6, 125.6, 124.9, 105.9, 85.3, 31.5, 28.2; HRMS (ESI) calcd for C$_{18}$H$_{25}$N$_3$O$_2$ (M$^+$) 316.2020, found 316.2018.

tert-butyl 2-amino-4-(2-nitrophenyl)-1H-imidazole-1-carboxylate (3.9b): In a similar manner as tert-butyl 2-amino-4-(4-butyphenyl)-1H-imidazole-1-carboxylate (3.3b), 0.400 g (1.64 mmol) of 2-bromo-1-(2-nitrophenyl)ethanone (3.9a) afforded 0.100 g (20%) of the title compound as a green solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.71 (d, 1H, $J = 10.6$ Hz), 7.63 (d, 1H, $J = 8.4$ Hz), 7.52 (t, 1H, $J = 10.0$ Hz), 7.37 (t, 1H, $J = 10.2$ Hz), 7.03 (s, 1H), 1.60 (s,
170

$\text{^13C NMR (100 MHz, CDCl}_3 \delta 150.5, 149.2, 133.1, 131.8, 130.5, 128.2, 127.4, 123.7, 109.4, 85.8, 28.1; HRMS (ESI) calcd for C}_{14}\text{H}_{16}\text{N}_4\text{O}_4 (M^+) 305.1244, found 305.1245.}$

$\text{tert-butyl 2-amino-4-(biphenyl-4-yl)-1H-imidazole-1-carboxylate (3.10b): In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 0.400 g (1.50 mmol) of 1-(biphenyl-4-yl)-2-bromoethanone (3.10a) afforded 0.094 g (19%) of the title compound as a yellow solid: $\text{^1H NMR (400 MHz, CDCl}_3 \delta 7.77 (d, 2H, J = 8.0 Hz), 7.62 (t, 3H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.2 Hz), 7.34 (d, 2H, J = 6.8 Hz), 7.15 (s, 1H), 6.12 (s, 2H), 1.64 (s, 9H);}$ $\text{^13C NMR (100 MHz, CDCl}_3 \delta 150.9, 141.0, 140.1, 137.6, 132.4, 128.9, 127.4, 127.1, 125.6, 106.5, 85.4, 28.2; HRMS (ESI) calcd for C}_{20}\text{H}_{21}\text{N}_3\text{O}_2 (M^+) 336.1707, found 336.1711.}$

$\text{tert-butyl 2-amino-4-(naphthalen-2-yl)-1H-imidazole-1-carboxylate (3.11b): In a similar manner as tert-butyl 2-amino-4-(4-butylphenyl)-1H-imidazole-1-carboxylate (3.3b), 0.400 g (1.60 mmol) of 2-bromo-1-(naphthalen-2-yl)ethanone (3.11a) afforded 0.031 g (6%) of the title compound as a tan solid:}$ $\text{^1H NMR (400 MHz, CDCl}_3 \delta 8.2 (s, 1H), 7.83 (m, 4H), 7.43}$
(m, 2H), 7.21 (s, 1H), 6.03 (s, 2H), 1.61 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.9, 149.6, 137.8, 133.9, 133.0, 128.4, 128.2, 128.1, 127.8, 127.4, 126.4, 125.9, 123.8, 123.6, 107.0, 85.5, 28.2; HRMS (ESI) calcd for C$_{18}$H$_{19}$N$_3$O$_2$ (M$^+$) 310.1550, found 310.1549.

4-(4-butylyphenyl)-1H-imidazol-2-amine hydrochloride (3.3c): tert-butyl 2-amino-4-(4-butylyphenyl)-1H-imidazole-1-carboxylate (3.3b) (0.151 g, 0.48 mmol) was dissolved in 2.0 mL of DCM and stirred. To the solution, TFA (0.90 mL) was added drop-wise, and the reaction was stirred at ambient temperature for 2 hours. The solution was concentrated to dryness, then the oil was dissolved in MeOH (2.0 mL) and concentrated HCl (0.3 mL) was added drop-wise and concentrated to dryness again. The solid was dissolved in Et$_2$O (7.0 mL), sonicated, the Et$_2$O was decanted off, and then concentrated to dryness. This afforded the title compound (0.118 g, 98%) as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) δ 7.46 (d, 2H, $J = 8.4$ Hz), 7.20 (d, 2H, $J = 8.4$ Hz), 7.08 (s, 1H), 2.57 (t, 2H, $J = 7.6$ Hz), 1.55 (quintet, 2H, $J = 7.6$ Hz), 1.32 (sextet, 2H, $J = 7.6$ Hz), 0.90 (t, 3H, $J = 7.2$ Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 149.1, 144.7, 130.2, 129.1, 126.3, 125.6, 109.3, 36.4, 34.8, 23.4, 14.4; HRMS (ESI) calcd for C$_{13}$H$_{17}$N$_3$ (M$^+$) 216.1495, found 216.1498.
4-(4-hexylphenyl)-1H-imidazol-2-amine hydrochloride (3.4c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.123 g (0.36 mmol) of tert-butyl 2-amino-4-(4-hexylphenyl)-1H-imidazole-1-carboxylate (3.4b) afforded 0.051 g (51%) of the title compound as a white solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.47 (d, 2H, $J = 8.4$ Hz), 7.22 (d, 2H, $J = 8.4$ Hz), 7.09 (s, 1H), 2.60 (t, 2H, $J = 8.0$ Hz), 1.59 (m, 2H), 1.30 (bs, 6H), 0.88 (t, 3H, $J = 6.8$ Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 149.2, 144.9, 130.3, 129.2, 126.4, 125.6, 109.3, 36.7, 32.9, 32.6, 30.1, 23.8, 14.6; HRMS (ESI) calcd for C$_{15}$H$_{21}$N$_3$ (M$^+$) 244.1808, found 244.1807.

4-(4-heptylphenyl)-1H-imidazol-2-amine hydrochloride (3.5c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.100 g (0.28 mmol) of tert-butyl 2-amino-4-(4-heptylphenyl)-1H-imidazole-1-carboxylate (3.5b) afforded 0.072 g (88%) of the title compound as a yellow solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.48 (d, 2H, $J = 8.0$ Hz), 7.23 (d, 2H, $J = 8.4$ Hz), 7.09 (s, 1H), 2.61 (t, 2H, $J = 7.6$ Hz), 1.60 (bs, 2H), 1.31 (bs, 8H), 0.88 (t, 3H, $J = 6.4$ Hz); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 149.1, 143.6, 129.0,
127.9, 126.8, 125.1, 124.4, 108.1, 35.4, 31.8, 31.3, 29.1, 22.5, 13.3; HRMS (ESI) calcd for C_{16}H_{23}N_{3} (M^+) 258.1965, found 258.1962.

4-(4-nonylphenyl)-1H-imidazol-2-amine hydrochloride (3.6c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.116 g (0.30 mmol) of tert-butyl 2-amino-4-(4-nonylphenyl)-1H-imidazole-1-carboxylate (3.6b) afforded 0.074 g (77%) of the title compound as a yellow solid: \( ^1H \) NMR (400 MHz, CD_{3}OD) \( \delta \) 7.47 (d, 2H, \( J = 8.4 \) Hz), 7.21 (d, 2H, \( J = 8.0 \) Hz), 7.09 (s, 1H), 2.59 (t, 2H, \( J = 7.2 \) Hz), 1.59 (bs, 2H), 1.30 (bs, 12H), 0.87 (t, 3H, \( J = 6.8 \) Hz); \( ^{13}C \) NMR (100 MHz, CD_{3}OD) \( \delta \) 149.3, 144.8, 130.3, 129.1, 126.4, 125.6, 109.3, 36.7, 33.2, 32.6, 30.8, 30.7, 30.5, 30.4, 23.8, 14.6; HRMS (ESI) calcd for C_{18}H_{27}N_{3} (M^+) 286.2278, found 286.2276.

4-phenyl-1H-imidazol-2-amine hydrochloride (3.7c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.064 g (0.25 mmol) of tert-butyl
2-amino-4-phenyl-1\textit{H}-imidazole-1-carboxylate (3.7b) afforded 0.039 g (80\%) of the title compound as a tan solid: \(^1\)H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta\) 7.56 (d, 2H, \(J = 8.8\) Hz), 7.42, (t, 2H, \(J = 7.2\) Hz), 7.34 (d, 1H, \(J = 8.4\) Hz), 7.14 (s, 1H); \(^1^3\)C NMR (100 MHz, CD\textsubscript{3}OD) \(\delta\) 149.5, 135.5, 130.3, 130.2, 129.7, 129.5, 129.0, 128.4, 125.7, 109.9; HRMS (ESI) calefd for C\textsubscript{9}H\textsubscript{9}N\textsubscript{3} (M\textsuperscript{+}) 160.0869, found 160.0865.

4-(4-\textit{tert}-butylphenyl)-1\textit{H}-imidazol-2-amine hydrochloride (3.8c): In a similar manner as 4-(4-butylphenyl)-1\textit{H}-imidazol-2-amine hydrochloride (3.3c), 0.060 g (0.250 mmol) of \textit{tert}-butyl 2-amino-4-(4-\textit{tert}-butylphenyl)-1\textit{H}-imidazole-1-carboxylate (3.8b) afforded 0.044 g (70\%) of the title compound as a yellow solid: \(^1\)H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta\) 7.46 (d, 2H, \(J = 8.8\) Hz), 7.41 (d, 2H, \(J = 8.0\) Hz), 7.07 (s, 1H), 1.28 (s, 9H); \(^1^3\)C NMR (100 MHz, CD\textsubscript{3}OD) \(\delta\) 153.0, 149.3, 148.8, 144.5, 129.1, 127.2, 126.1, 125.5, 109.4, 35.7, 31.7; HRMS (ESI) calefd for C\textsubscript{13}H\textsubscript{17}N\textsubscript{3} (M\textsuperscript{+}) 216.1495, found 216.1497.

4-(2-nitrophenyl)-1\textit{H}-imidazol-2-amine hydrochloride (3.9c): In a similar manner as 4-(4-butylphenyl)-1\textit{H}-imidazol-2-amine hydrochloride (3.3c), 0.067 g (0.220 mmol) of \textit{tert}-butyl
2-amino-4-(2-nitrophenyl)-1H-imidazole-1-carboxylate (3.9b) afforded 0.053 g (100%) of the title compound as a brown solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.11 (d, 1H, $J = 8.0$ Hz), 7.79 (t, 1H, $J = 7.6$ Hz), 7.68 (m, 2H), 6.99 (s, 1H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 149.5, 134.8, 134.5, 133.6, 131.9, 126.2, 124.5, 123.5, 113.2; HRMS (ESI) calcd for C$_9$H$_8$N$_4$O$_2$ (M$^+$) 205.0720, found 205.0725.

4-(biphenyl-4-yl)-1H-imidazol-2-amine hydrochloride (3.10c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.058 g (0.170 mmol) of tert-butyl 2-amino-4-(biphenyl-4-yl)-1H-imidazole-1-carboxylate (3.10b) afforded 0.045 g (96%) of the title compound as a tan solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.69 (s, 1H), 7.62 (m, 5H), 7.43 (m, 2H), 7.34 (m, 2H), 7.18 (s, 1H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 149.4, 142.6, 141.4, 130.1, 128.9, 128.7, 127.9, 126.1, 110.1; HRMS (ESI) calcd for C$_{15}$H$_{13}$N$_3$ (M$^+$) 236.1182, found 236.1183.
4-(naphthalen-2-yl)-1H-imidazol-2-amine hydrochloride (3.11c): In a similar manner as 4-(4-butylphenyl)-1H-imidazol-2-amine hydrochloride (3.3c), 0.026 g (0.080 mmol) of tert-butyl 2-amino-4-(naphthalen-2-yl)-1H-imidazole-1-carboxylate (3.11b) afforded 0.021 g (100%) of the title compound as a yellow solid: \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 8.00 (s, 1H), 7.85 (m, 2H), 7.63 (d, 2H, \(J = 8.4\) Hz), 7.50 (m, 2H), 7.24 (s, 1H); \(^13\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) 134.9, 134.5, 130.2, 129.2, 128.9, 128.1, 127.8, 127.5, 126.3, 124.5, 124.0, 123.5, 110.6; HRMS (ESI) calcd for C\(_{13}\)H\(_{11}\)N\(_3\) (M\(^+\)) 210.1026, found 210.1030.

N-Decyl-4-pentyl-benzamide (3.12): Decylamine (0.500 g, 3.20 mmol) was dissolved in 10 mL of DCM. TEA (0.648 g, 6.40 mmol) was added to the solution at 0 °C and allowed to stir for 5 min. and then 4-pentyl benzoyl chloride (0.809 g, 3.84 mmol) was added dropwise to the round bottom flask and remained stirring at 0 °C for an additional 15 min. The reaction was then stirred for an additional 2 h at ambient temperature. The reaction was then quenched with 30 mL of H\(_2\)O and partitioned with EtOAc (40 mL x 2), then the EtOAc was rinsed with brine (30 mL), dried with Na\(_2\)SO\(_4\), filtered, and concentrated to dryness. The crude solid was then purified via flash column chromatography (5 – 20% EtOAc:Hex) to afford 0.934 g (88%) of the title compound as a white solid: \(^1\)H NMR (300 MHz, DMSO) \(\delta\) 8.33 (t, 1H, \(J = 5.7\) Hz), 7.73 (d, 2H, \(J = 8.1\) Hz), 7.24 (d, 2H, \(J = 8.4\) Hz), 3.22 (q, 2H, \(J = 6.6, 12.9\) Hz), 2.60 (t, 2H, \(J = 7.5\) Hz), 1.54 (m, 4H), 1.26 (m, 20H), 0.84 (m, 6H); \(^13\)C NMR
(75 MHz, CDCl$_3$) δ 167.7, 146.6, 132.3, 128.5, 127.1, 40.2, 35.9, 32.0, 31.5, 31.0, 29.8, 29.7, 29.5, 29.4, 27.2, 22.8, 22.6, 14.2, 14.1; HMRS (ESI) calcd for C$_{22}$H$_{37}$NO (M$^+$) 332.2948, found 332.2946.

N-Benzyl-4-pentyl-benzamide (3.13): Following the same procedure as N-Decyl-4-pentyl-benzamide (3.12), 0.500 g (4.70 mmol) of benzylamine and 1.18 g (5.60 mmol) of 4-pentyl benzoyl chloride afforded 0.744 g (59%) of the title compound as a white powder: $^1$H NMR (300 MHz, DMSO) δ 0.89 (t, 1H, $J = 5.7$ Hz), 7.82 (d, 2H, $J = 7.8$ Hz), 7.26 (m, 7H), 4.47 (d, 2H, $J = 6.0$ Hz), 2.62 (t, 2H, $J = 7.2$ Hz), 1.56 (t, 2H, $J = 7.5$ Hz), 1.29 (m, 4H), 0.85 (t, 3H, $J = 6.9$ Hz); $^{13}$C NMR (75 MHZ, CDCl$_3$) δ 167.6, 146.9, 138.6, 131.8, 128.7, 128.6, 127.9, 127.5, 127.2, 44.0, 35.9, 31.5, 31.0, 22.6, 14.1; HRMS (ESI) calcd for C$_{19}$H$_{23}$NO (M$^+$) 282.1852, found 282.1854.

N-(4-Methoxy-benzyl)-4-pentyl-benzamide (3.14): Following the same procedure as as N-Decyl-4-pentyl-benzamide (3.12), 0.500 g (3.60 mmol) of 4-methoxybenzylamine and 0.927 g (4.40 mmol) of 4-pentyl benzoyl chloride afforded 1.06 g (95%) of the title compound as a
white solid: $^1$H NMR (300 MHz, DMSO) $\delta$ 8.88 (t, 1H, $J = 6.0$ Hz), 7.82 (t, 2H, $J = 8.4$ Hz), 7.25 (m, 4H), 6.87 (m, 2H), 4.41 (d, 2H, $J = 6.0$ Hz), 3.72 (s, 3H), 2.61 (t, 2H, $J = 7.2$ Hz), 1.55 (m, 2H), 1.24 (m, 4H), 0.85 (t, 3H, $J = 7.2$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 167.5, 158.9, 146.7, 131.8, 130.7, 129.1, 128.5, 127.2, 114.0, 55.2, 43.4, 35.8, 31.4, 30.9, 22.5, 14.1; HRMS (ESI) calcd for C$_{20}$H$_{25}$NO$_2$ (M$^+$) 312.1958, found 312.1963.

$N$-(4-Bromo-phenyl)-4-pentyl-benzamide (3.15): Following the same procedure as as $N$-Decyl-4-pentyl-benzamide (3.12), 0.500 g (2.90 mmol) of 4-bromoaniline and 0.737 g (3.50 mmol) of 4-pentyl benzoyl chloride afforded 0.130 g (14%) of the title compound as a tan solid: $^1$H NMR (300 MHz, DMSO) $\delta$ $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.2, 147.2, 139.4, 132.815, 132.1, 129.0, 128.4, 122.8, 115.9, 35.6, 31.5, 31.1, 22.6, 14.6; HRMS (ESI) calcd for C$_{18}$H$_{20}$BrNO (M$^+$) 346.0801, found 346.0804.

$N$-(4-Bromo-benzyl)-4-pentyl-benzamide (3.16): Following the same procedure as as $N$-Decyl-4-pentyl-benzamide (3.12), 0.090 g (0.47 mmol) of 4-bromobenzylamine and 0.100 g (0.47 mmol) of 4-pentyl benzoyl chloride afforded 0.158 g (95%) of the title compound as a
white solid: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.6 (d, 2H, $J = 8.4$ Hz), 7.34 (d, 2H, $J = 8.1$ Hz), 7.08 (m, 4H), 6.78 (bs, 1H), 4.41 (s, 2H), 2.53 (t, 2H, $J = 7.5$ Hz), 1.48 (m, 2H), 1.23 (m, 4H), 0.78 (t, 3H, $J = 5.4$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.7, 147.2, 137.7, 131.6, 131.9, 129.6, 128.8, 127.2, 121.4, 43.4, 35.9, 31.0, 22.6, 14.2; HRMS (ESI) calcd for C$_{19}$H$_{23}$BrNO (M$^+$) 360.0958, found 360.0959.

![Image](image1.png)

**N-(4-Methoxy-benzyl)-4-pentyl-benzamide (3.17):** Following the same procedure as as N-Decyl-4-pentyl-benzamide (3.12), 0.072 g (0.47 mmol) of 2-(4-methoxyphenyl)ethylamine and 0.100 g (0.47 mmol) of 4-pentyl benzoyl chloride afforded 0.150 g (97%) of the titles compound as a white solid: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.53 (d, 2H, $J = 7.8$ Hz), 7.10 (d, 2H, $J = 8.4$ Hz), 7.03 (d, 2H, $J = 8.4$ Hz), 6.75 (d, 2H, $J = 8.7$ Hz), 6.28 (bs, 1H), 3.69 (s, 3H), 3.55 (q, 2H, $J = 6.0$, 12.9 Hz), 2.76 (t, 2H, $J = 6.9$ Hz), 2.53 (t, 2H, $J = 7.5$ Hz), 1.49 (m, 2H), 1.21 (m, 4H), 0.80 (t, 3H, $J = 6.6$ Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.6, 158.4, 146.8, 132.2, 131.1, 129.9, 128.7, 127.0, 114.2, 55.4, 41.4, 35.9, 35.0, 31.5, 31.0, 22.6, 14.1; HRMS (ESI) calcd for C$_{21}$H$_{28}$NO$_2$ (M$^+$) 326.2115, found 326.2112.

![Image](image2.png)
4-Pentyl-benzoic acid cyclohexyl ester (3.18): Cyclohexanol (0.500 g, 5.00 mmol) was dissolved in a round bottom flask in 10 mL of DCM under N\textsubscript{2}. TEA (1.00 g, 9.98 mmol) was added to the reaction, followed by DMAP (cat.). Lastly, 4-pentyl benzoyl chloride (1.26 g, 5.99 mmol) was added dropwise to the round bottom flask and stirred at ambient temperature overnight. The reaction was rinsed with EtOAc (20 mL) and H\textsubscript{2}O (20 mL), and the EtOAc layer was rinsed with brine (30 mL), dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and concentrated to dryness. The crude oil was purified via flash column chromatography (5 – 10% EtOAc:Hex) affording 0.412 g (30%) of the title compound as a yellow oil: \textsuperscript{1}H NMR (300 MHz, DMSO) δ 8.00 (d, 2H, \textit{J} = 8.1 Hz), 7.24 (d, 2H, \textit{J} = 8.4 Hz), 2.64 (t, 2H, \textit{J} = 7.2 Hz), 1.90 (bs, 2H), 1.62 (m, 4H), 1.27 (m, 8H), 0.87 (t, 3H, \textit{J} = 6.6 Hz); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 171.7, 149.4, 130.4, 128.6, 127.3, 70.6, 36.2, 35.5, 31.6, 31.0, 25.6, 24.3, 22.7, 14.1.

![Structure of 4-Pentyl-benzoic acid phenyl ester](image)

4-Pentyl-benzoic acid phenyl ester (3.19): Following the same procedure as 4-Pentyl-benzoic acid cyclohexyl ester (3.18), 0.090 g (0.94 mmol) of phenol and 0.200 g (0.94 mmol) of 4-pentyl benzoyl chloride afforded 0.214 g (84%) of the title compound as a yellow oil: \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 8.15 (d, 2H, \textit{J} = 8.1 Hz), 7.44 (t, 2H, \textit{J} = 6.6 Hz), 7.32 (d, 2H, \textit{J} = 8.4 Hz), 7.24 (m, 2H), 2.71 (t, 2H, \textit{J} = 7.2 Hz), 1.65 (m, 2H), 1.34 (m, 4H), 0.93 (t, 3H, \textit{J} = 6.9 Hz); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) δ 165.4, 151.2, 149.5, 130.4, 129.6, 128.8, 127.2,
125.9, 121.9, 36.2, 31.6, 31.0, 22.7, 14.2; HRMS (ESI) calcd for C\textsubscript{18}H\textsubscript{20}O\textsubscript{2} (M\textsuperscript{+}) 269.1536, found 269.1537.

4-Pentyl-benzoic acid benzyl ester (3.20): Following the same procedure as 4-Pentyl-benzoic acid cyclohexyl ester (3.18), 0.102 g (0.94 mmol) of benzyl alcohol and 0.200 g (0.94 mmol) of 4-pentyl benzoyl chloride afforded 0.254 g (95%) of the title compound as a yellow oil: \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 7.90 (d, 2H, \(J = 8.4\) Hz), 7.34 (m, 2H), 7.27 (m, 3H), 7.15 (m, 2H), 5.25 (s, 2H), 2.54 (t, 2H, \(J = 7.2\) Hz), 1.50 (m, 2H), 1.20 (m, 4H), 0.79 (t, 3H, \(J = 6.9\) Hz); \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 166.6, 148.8, 136.4, 129.9, 128.7, 128.6, 128.3, 128.3, 127.8, 66.6, 36.1, 31.6, 31.0, 22.6, 14.1; HRMS (ESI) calcd for C\textsubscript{19}H\textsubscript{20}O\textsubscript{2} (M\textsuperscript{+}) 305.1512, found 305.1512.

4-Pentyl-benzoic acide propyl phenyl ester (3.21): Following the same procedure as 4-Pentyl-benzoic acid cyclohexyl ester (3.18), 0.500 g (3.60 mmol) of 3-phenyl-1-propanol and 0.928 g (4.40 mmol) afforded 0.995 g (89%) of the title compound as a clear oil: \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 7.85 (d, 2H, \(J = 8.4\) Hz), 7.23 (m, 7H), 4.22 (t, 2H, \(J = 6.3\) Hz), 2.70 (t,
2H, \( J = 6.6 \text{ Hz} \), 2.59 (t, 2H, \( J = 7.5 \text{ Hz} \)), 1.98 (m, 2H), 1.51 (m, 2H), 1.23 (m, 4H), 0.81 (t, 3H, \( J = 6.9 \text{ Hz} \)); \(^{13}\text{C NMR} (75 \text{ MHz, CDCl}_3) \delta 165.6, 148.5, 141.1, 129.2, 128.5, 128.3, 127.3, 125.8, 63.7, 35.1, 31.6, 30.8, 30.3, 29.8, 21.9, 13.8; \text{HRMS (ESI) calcd for C}_{21}\text{H}_{26}\text{O}_2 (M^+) \text{311.2006, found 311.2004.}

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\begin{align*}
\text{3.22}
\end{align*}
\]

**4-(4-pentylphenyl)thiazol-2-amine (3.22):** 2-bromo-1-(4-pentylphenyl)ethanone (3.3a) (0.400 g, 1.50 mmol) was dissolved in EtOH (10 mL). Thiourea (0.114g, 1.50 mmol) was added to the solution, and stirred at 90 °C for 4 hours. After completion, the reaction was cooled to room temperature and quenched with ammonium hydroxide until product crashes out of solution. The solid product was filtered out with H2O and then recrystallized in hexanes to afford 0.201 g (54%) of 4-(4-pentylphenyl)thiazol-2-amine as a white solid: \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3) \delta 7.71 \text{ (d, 2H, } J = 8.0 \text{ Hz)}, 7.22 \text{ (d, 2H, } J = 8.4 \text{ Hz)}, 6.63 \text{ (s, 1H)}, 2.65 \text{ (t, 2H, } J = 7.2 \text{ Hz)}, 1.68 \text{ (bs, 2H)}, 1.39 \text{ (bs, 4H)}, 0.96 \text{ (t, 3H, } J = 6.8 \text{ Hz}); \(^{13}\text{C NMR} (100 \text{ MHz, CDCl}_3) \delta 178.5, 161.3, 152.7, 142.5, 138.9, 136.1, 111.7, 35.7, 31.5, 31.1, 22.6, 14.1; \text{HRMS (ESI) calcd for C}_{14}\text{H}_{18}\text{N}_2\text{S (M^+) 247.1263, found 247.1263.}

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\begin{align*}
\text{3.23}
\end{align*}
\]
2-(4-pentyl-phenyl)-4,5-dihydro-oxazole (3.23): 2-Bromoethylamine hydrobromide (0.369 g, 1.80 mmol) was dissolved in toluene (6.5 mL) in a round bottom flask. To this solution, TEA (0.911g, 9.0 mmo) was added and the solution was stirred for 5 min. 4-pentyl benzoyl chloride (0.414g, 2.0 mmol) was added dropwise to the round bottom flask and stirred at ambient temperature for 2 h, then at 135 °C for 22 h. The round bottom flask was cooled to room temperature, rinsed with EtOAc (20 mL) and H2O (15 mL). The organic layer was extracted, dried (Na2SO4), filtered, and concentrated to dryness. The crude oil was then purified via flash column chromatography (5-15% EtOAc:Hexanes) to afford 0.379 g (97%) of the title compound as a clear oil: 1H NMR (400 MHz, CDCl3) δ 7.84 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz), 4.42 (t, 2H, J = 9.6 Hz), 4.05 (t, 2H, J = 9.6 Hz), 2.63 (t, 2H, J = 7.2 Hz), 1.62 (t, 2H, J = 7.2 Hz), 1.32 (t, 4H, J = 3.2 Hz), 0.88 (t, 3H, J = 6.8 Hz); 13C NMR (100 MHz, CDCl3) δ 147.2, 130.3, 129.1, 128.9, 128.7, 127.5, 67.3, 55.3, 36.3, 31.4, 30.5, 23.0, 14.5; HRMS (ESI) calcd for C14H19NO (M+) 218.1539, found 218.1536.

Biological Screening Experimental

Microdilution Susceptibility Testing (MIC Screens)

All antibiotic susceptibility testing was completed with a starting inoculum of 5 x 10^5 CFU mL^-1 according to NCCLS41 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 even in the absence of turbidity.

Microdilutions were made in 96 well microtiter plates from a starting concentration of 400 µM with the addition of 200 µL to a predetermined well. From this, twofold 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 pipet, this next well then contained 200 µL of bacterial, media and test 2ABI at a concentration of 200 µM. This transfer of 100 µL was done in succession for a total of 10 two-fold serial dilutions giving a range of 400 µM–391 nM for tested antibiotic in 96 well microtiter plates. The final dilution then had 200 µL (of the 391 nM wells) and then 100 µL was removed and thrown away. A plastic lid was then used to cover the wells and the plates with their lids were wrapped in Saran wrap and placed in a humidified chamber and incubated for 16–20 h at 37 °C. In one plate, 8 antibiotics could be run in parallel with 10 two-fold dilutions. After the incubation time, the wells were observed for bacterial growth and MICs were determined accordingly (described above).

**Procedure to Determine the Inhibition Effect of Test Compounds on A. baumannii and E. coli Biofilms:** Inhibition assays were performed by taking an overnight culture and sub-culturing it with an OD$_{600}$ of 0.01 into the necessary media; Luria-Mertanit (LB) for *A. baumannii* and *E. coli*. Stock solutions of predetermined concentrations of the test compounds were then made with DMSO (biology grade). These stock solutions were aliquoted (100 µL) into the wells of a 96-well PVC microtiter plate. Sample plates were wrapped in GLAD Press
n’ Seal® followed by incubation under stationary conditions for 24 h at 37 °C. After incubation the media was discarded and the plates were washed with water. The sample plates were then stained with 110 µL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. The CV stain was then discarded and the plates were washed with water. The remaining stain was solubilized with 200 µL of 95% ethanol. After the biofilms were dissolved (5 minutes) a sample of 125 µL of solublized CV stained ethanol was transferred from each well into the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD$_{540}$ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out. The percent inhibition was calculated by the comparison of the OD$_{540}$ for established biofilm (control) versus treated established biofilm (compound treated) under identical conditions.

**Procedure to Determine the Dispersal Effect of Test Compounds on E. coli Preformed Biofilms:** Overnight cultures of *A. baumannii* and *E. coli* bacteria were subcultured at an OD$_{600}$ of 0.05 into LB media and then aliquoted (100 µL) into a 96 well plate. The 96 well plates were then covered with GLAD Press n’ Seal® and incubated at 37 °C for 24 hours. After 24 hours the medium was discarded and the plate was washed with water. Then fresh media with the appropriate concentration of compound was added to the wells. The plate was then covered again in the same manner and placed in the 37 °C oven for 24 hours. After 24 hours the media was discarded and the plate was washed with water. The wells were then
incubated with 0.1% of CV (110 µL) and allowed to stand at ambient temperature for 30 minutes. After which the CV was discarded and the plate was thoroughly washed with water. Then the remaining stain was solubilized was 200 µL of 95% ethanol and allowed to stand for 5 minutes. Biofilm dispersion was quantified by measuring the OD$_{540}$ for each well by transferring 125 µL of CV stained ethanol. The percent dispersion was calculated the same way as for inhibition.

REFERENCES


APPENDICES
NMR Spectra

![NMR Spectra Image]

188
3.8a

Pulse Sequence: 35ps
Sample: 4.000 MHz
Power: 40.0 W
Pulse: 90° pulse
Data: 1000 points
Data: 48000 points
Time: 1.5 min
Total time: 25 min, 15 sec

[Chemical structure image]
3.18
Dispersion Dose Response Curves

\[ E. \textit{coli} \text{ K-12 ER2738} \]

![Dispersion Dose Response Curve](image)

% Dispersion vs Concentration (µM)
E. coli K-12 ER2738

Concentration (µM)

% Dispersion

E. coli K-12 ER2738

Concentration (µM)

% Dispersion

A. baumannii

Concentration (µM)

% Dispersion

245