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Property-Guided Synthesis of Tricyclic Indolines to Confront Antibiotic Resistance in Methicillin Resistant Staphylococcus aureus

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PROPERTY-GUIDED SYNTHESIS OF TRICYCLIC INDOLINES TO CONFRONT ANTIBIOTIC RESISTANCE IN METHICILLIN RESISTANT STAPYLOCOCCUS AUREUS

by

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written by Patrick Michael Barbour
has been approved for the Department of Chemistry and Biochemistry

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Date: ______________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
ABSTRACT

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PROPERTY-GUIDED SYNTHESIS OF TRICYCLIC INDOLINES TO CONFRONT
ANTIBIOTIC RESISTANCE IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Thesis directed by Professor Xiang Wang

Antibiotics are essential to modern medicine. They have enabled surgery to become routine and allowed for the development of advanced treatments for cancer and burn patients. However, a reservoir of genes that confer resistance and aid in host colonization is distributed across the microbial world and decreases the ability of modern medicine to control infection. We have not been able to sustainably counteract this with new drugs and resistance has been observed for all antibiotics that have been employed in the clinic. New classes of antibiotics are needed, but we also need to explore ways to restore the efficacy of antibiotics made obsolete because of resistance.

A variety of small-molecules can be used to reverse antibiotic resistance; these compounds have been named resistance modifying agents or RMAs. In this dissertation, a summary of antibiotic resistance will be presented alongside antibiotic drug discovery strategies. These topics will highlight the importance of my research to address antibiotic resistance in S. aureus with tricyclic indoline alkaloids.

In Chapter 2, I will report the discovery of N-benzyl tricyclic indolines as antibiotics with activity in methicillin sensitive S. aureus (MSSA) and multiple methicillin resistant S. aureus
(MRSA) strains. Then I will discuss the structure-activity relationship (SAR) study that led to a more potent analogue of this antibiotic class.

In Chapter 3, I will discuss the property-guided synthesis of a novel tricyclic indoline RMA that is capable of re-sensitizing MRSA to methicillin and other β-lactam antibiotics. These RMAs have a unique aza-tricyclic indoline (ATI) core structure that was inspired by natural products and cLogD\textsubscript{7.4} calculations. To synthesize ATIs, we developed a new synthetic route that incorporated a robust gold-catalyzed tandem cyclization. This new route has led to a potent RMA for β-lactam antibiotics with the ATI core. This RMA has excellent physical properties and is now a candidate for preclinical studies.
DEDICATION

To all my teachers past and present
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# TABLE OF CONTENTS

LIST OF TABLES...........................................................................................................ix

LIST OF FIGURES................................................................................................................x

LIST OF SCHEMES...........................................................................................................xi

LIST OF ABBREVIATIONS...............................................................................................xii

1. INTRODUCTION

1.1. Ancient Antibiotic Resistance................................................................................1

1.2. Antibiotic Resistance in Modern Healthcare.........................................................2

1.3. Obstacles to Modern Antibiotic Drug Discovery...................................................3

1.4. MRSA Resistance Mechanisms that Target β-Lactam Antibiotics............................4

1.5. Overcoming Resistance to β-lactam Antibiotics with Medicinal Chemistry..............5

1.6. β-Lactamase inhibitors, the First Resistance Modifying Agents..............................7

1.7. Overview of Dissertation..........................................................................................8

2. DISCOVERY AND INITIAL STRUCTURE-ACTIVITY RELATIONSHIPS OF N-BENZYL
   TRICYCLIC INDOLINES AS ANTIBACTERIALS FOR METHICILLIN RESISTANT
   STAPHYLOCOCCUS AUREUS

2.1. Introduction..............................................................................................................10
2.2. Results and Discussion ..................................................................................14

2.3. Conclusions .................................................................................................20

2.4. Outlook ........................................................................................................21

2.5. Experimental Methods ................................................................................22

TABLE OF CONTENTS

3. PROPERTY-GUIDED SYNTHESIS OF AZA-TRICYCLIC INDOLINES:

DEVELOPMENT OF GOLD CATALYSIS EN ROUTE

3.1. Introduction ...................................................................................................29

3.2. Results and Discussion ................................................................................36

3.3. Conclusions ..................................................................................................49

3.4. Outlook .........................................................................................................51

3.5. Experimental Methods ................................................................................51

BIBLIOGRAPHY .................................................................................................88

APPENDIX

A.1 Biological and Physiochemical Assay Protocols and Results .......................102

A.2 Nuclear Magnetic Resonance Spectra ..........................................................109
LIST OF TABLES

2.1 Structures and MIC values of Of4 analogues against MSSA.................................17
2.2 Evaluation of the MIC values of 4k in a panel of MRSA strains..............................19
3.1 Optimization of the tandem cyclization reaction..................................................39
3.2 Scope of gold-catalyzed tandem cyclization.........................................................43
3.3 Comparison of the biological activity of Of1 and ATIs..........................................46
S.1 Aza-Tricyclic Indoline Aqueous Solubility.............................................................106
# LIST OF FIGURES

1.1 Medicinal Chemistry Optimization of $\beta$-Lactam Antibiotics .................................................. 6  
1.2 Structures of $\beta$-Lactamase Inhibitors ......................................................................................... 8  
2.1 Synthesis of Bio-Inspired Indole Alkaloids with Diverse Biological Activity ...................... 13  
2.2 Determination of GI$_{50}$ for 4K in HeLa Cells ................................................................................... 18  
2.3 Future SAR study for $N$-benzyl Tricyclic Indoline Antibiotics ................................................ 21  
3.1 Survey of RMAs for $\beta$-Lactam Antibiotics ................................................................................. 30  
3.2 The BlaR/I Signaling Pathway in MRSA ....................................................................................... 32  
3.3 Design and Proposed Targeted Synthesis of Aza-Tricyclic Indolines ....................................... 35  
3.4 Single-Dose Pharmacokinetic Study of the Aza-Tricyclic Indoline 13 ...................................... 48  
S1 Phase I Metabolic Stability of Aza-Tricyclic Indolines and Tricyclic Indolines ................ 105
LIST OF SCHEMES

2.1 Synthesis of Of4 and Analogues 4a-k ................................................................. 15

3.1 Synthesis of the Cyclization Precursor 8a ............................................................. 36

3.2 Proposed Mechanism for Tandem Cyclization ...................................................... 40

3.3 Synthesis of Aza-Tricyclic Indolines ................................................................. 44
LIST OF ABBREVIATIONS

AcOH acetic acid
Amox/clav amoxicillin/clavulanic acid
ATCC American Type Culter Collection
ATI Aza-Tricyclic Indoline
AUC Area under the curve
BlaI bla operon transcriptional repressor
BlaR bla operon response regulator
Boc tert-butoxycarbonyl
Boc₂O tert-butoxycarbonyl anhydride
BPO benzoyl peroxide
CBz benzyloxy carbonyl
CDC Centers for Disease Control and Prevention
Cef cefazolin
Cf Closed-Fused-Tetracyclic-Indoline
cLogD_{7.4} calculated logarithm of distribution coefficient at pH 7.4
Cls 4-chloro-benzene-sulfonyl
CLSI Clinical and Laboratory Standards Institute
C_{max} maximum concentration
CNs 4-chloro-2-nitro-benzene-sulfonyl
Cs     Closed-Spiro-Tetracyclic-Indoline
DMAP   4-dimethyl-aminopyridine
DMEM   Dulbecco's modified Eagle's medium
DMSO   dimethyl sulfoxide
GI50   half growth inhibitory concentrations
HeLa   human cervical adenocarcinoma cells
HRMS   high-resolution mass spectrometry
IP     intraperitoneal
IPr    (2,6-diisopropylphenyl)imidazol-2-ylidene
Kf     Alkylated-Fused-Indoline
Ks     Alkylated-Spiro-Indoline
LC-MS  high performance liquid chromatography linked mass spectrometry
MecI   meC operon transcriptional repressor
MecR   meC operon response regulator
MHB    Mueller-Hinton Broth
MIC    Minimum Inhibitory Concentration
MRC    Minimum Resensitizing Concentration
MRSA   Methicillin Resistant *Staphylococcus aureus*
Ms     methanesulfonyl
MsOH   methane sulfonic acid
MSSA   Methicillin Sensitive *Staphylococcus aureus*
NARSA  The Network on Antimicrobial Resistance in *Staphylococcus aureus*
NBS    *N*-bromo succinimide
Ns     4-nitro-benzene-sulfonyl
NT     Not Tested
Of1  Opened-Fused-Tricyclic-Indoline 1
Of4  Opened-Fused-Tricyclic-Indoline 4
PBP2  Penicillin Binding Protein 2
PBP2a  Penicillin Binding Protein 2a
PK  Pharmacokinetics
RMA  Resistance Modifying Agent
SAR  Structure-Activity Relationship
T1/2  Half-Life of drug *in vivo*
Tfa  trifluoroacetamide
TFA  trifluoroacetic acid
TFAA  trifluoroacetic anhydride
TMB  3,4,5-trimethoxybenzoyl
TPP  triphenyl phosphine
Ts  4-methyl-benzene-sulfonyl
VraRS  Cell-wall stress response two-component system
VRSA  Vancomycin resistant *Staphylococcus aureus*
XPhos  2-dicyclohexylphosphino-2', 4', 6'-triisopropylbiphenyl
CHAPTER 1

INTRODUCTION

1.1 Ancient Antibiotic Resistance

Microorganisms have been competing with each other for the last 3.5 billion years. Their main tools for competition are antibiotics that can slow the growth of their competitors and provide an advantage in nutrient limited environments (Nesme and Simonet 2015). This selective evolutionary pressure in natural environments has led to parallel evolution of resistance mechanisms to block the action of antibiotics. In support of this, ancient resistance genes have been discovered in multiple studies that examine ancient “snapshots” of microbial life in ice cores (D’Costa et al. 2011) or anthropologically isolated caves (Bhullar et al. 2012). This reservoir of ancient resistance genes is named the resistome (Wright 2007). The presence of the resistome confirms that widespread clinical use of antibiotics in the 20th century did not cause resistance genes to develop, but rather, selected for organisms that can gain or utilize ancient genes that confer resistance to antibiotics.
1.2 Antibiotic Resistance in Modern Healthcare

The use of antibiotics in modern healthcare has fundamentally improved outcomes for surgical operations, cancer treatment and burn patients. For this reason the development of antibiotic resistance in pathogenic microorganisms remains an ongoing public health threat (Laxminarayan et al. 2013). According to the Centers for Disease Control and Prevention, there are roughly 2 million cases of infections caused by antibiotic resistant bacteria each year in the United States. Roughly 23,000 of these infections end with a fatality and methicillin-resistant *S. aureus* (MRSA) infections account for nearly half of these deaths (CDC 2013). MRSA is the most common hospital acquired pathogen, and we now have MRSA strains that are resistant to last-line-of-defense antibacterial agents such as: linezolid (Morales et al. 2010), daptomycin (Marty et al. 2006) and vancomycin (Hiramatsu et al. 1997; Hiramatsu 2001). This means that we have entered a period of modern healthcare where physicians may not have the tools to manage infection. To exacerbate the issue, research and development of novel antibiotics and other treatment options has been abandoned by most major pharmaceutical companies.

Antibiotic resistance has become such a concern that multiple organizations have released reports that highlight the current inadequacies of treating infectious disease in modern healthcare. The Infectious Diseases Society of America (ISDA) has issued a “call to arms” to bring antibiotic resistance into public discussion. (Boucher et al. 2009). Then in 2010, the ISDA posed a challenge to develop 10 new systemic antibiotics by the year 2020 in the so called “10’ X 20 Initiative”. Encouragingly, the Obama administration has responded by releasing a “National Action Plan for Combating Antibiotic-Resistant Bacteria”. This plan addresses antibiotic resistance by presenting multiple goals for policy makers and researchers alike. The
The most important goal of these reports is that the development of new therapies for the treatment of drug-resistant bacteria needs to continue in a sustainable way. These reports provide fresh impetus for antibiotic research and development that will spark innovation in antibiotic drug discovery. Even with this supportive outlook from policy makers, the discovery of new antibiotics with distinct mechanism of action is an arduous task that is not solved solely by increased expenditure of resources.

### 1.3 Obstacles to Modern Antibiotic Drug Discovery

The problem is that the antibiotics of natural origin discovered in the 1940s and 1960s were the low hanging fruit of the microbial world and attempts to discover additional natural product antibiotics led to re-discovery of known compounds. In addition, attempts to develop synthetic antibacterials have proven to be quite difficult as well. For example, in 1995 the first bacterial genome was sequenced, offering a ray of hope to synthetic antibiotic research and development. With a sequenced genome, researchers at the time rationalized that they could identify genes for essential biochemical pathways in bacteria. They could then develop *in vitro* assays for these pathways then screen large small-molecule libraries in a high-throughput format to discover new inhibitors. GlaxsoSmithKline has been the most vocal about their efforts to discover new antibiotics using this approach (Payne et al. 2007). In their seven year campaign they found nearly 300 genes that were vital for bacterial growth and performed 70 high-throughput screening efforts to discover antibiotic lead compounds. They spent close to 70 million dollars and found no viable leads to bring to pre-clinical testing. They attributed their difficulties in part to the sparse structural similarities between the compounds in their screening
libraries and the natural products that have been traditionally developed as antibiotics. This story describes in part the difficulties in developing new classes of antibiotics that has led major pharmaceutical companies to drop out of antibiotic research and development. However, with recent technological advances, natural product antibiotic isolation is regaining traction as a source of new antibiotic discovery (Wright 2014).

The prospect of discovering a new antibiotic class from natural sources is reassuring but the resistome predicts that resistance will develop for any antibiotic introduced to the clinic. This requires us to think about mechanisms of resistance to better understand how to overcome them. Our current knowledge shows that bacteria use three broadly defined types of resistance mechanisms to evade antibiotics: 1) they remove the antibiotic from the interior of the cell (Li and Nikaido 2009), 2) they deploy enzymes to destroy the antibiotic (Wright 2005), or 3) they modify the biological target of the antibiotic (Spratt 1994). These resistance mechanisms can be used simultaneously to allow bacteria to resist multiple classes of antibiotics. Due to the breadth of resistance mechanism for antibiotics, we will focus our discussion to specific resistance mechanisms for β-lactam containing antibiotics in MRSA, which rely on target modification and antibiotic destruction.

1.4 MRSA Resistance Mechanisms that Target β-Lactam Antibiotics

Penicillin was isolated from fungi that were observed to inhibit the growth of S. aureus cultures (Flemming 1932) and is the first member of the penem structural class of β-lactam containing antibiotics (Figure 1.1A). Penicillin was later found to inhibit penicillin binding protein 2 (PBP2). PBP2 is a vital cell-wall metabolic enzyme that maintains cell-wall structure
and prevents cell lysis. Penicillin inhibits growth by mimicking the natural substrate of this enzyme and irreversibly binding the active site (Tipper and Strominger 1965). In light of this, MRSA is resistant to β-lactam antibiotics by producing a modified version of PBP2 known as PBP2a. This enzyme is an example of a modified biological target for antibiotics (resistance mechanism number 3, see above); the active site of PBP2a can only be accessed through allosteric binding of a second ligand. This allows MRSA to continue cell-wall metabolism in the presence of high concentrations of penicillin or other penems (Otero et al. 2013). In addition to PBP2a, MRSA employs β-lactamase enzymes that specifically bind and destroy the β-lactam pharmacophore before it can inhibit PBP2 (Draws and Bonomo 2010) (resistance mechanism number 2, see above). β-Lactamases are especially troubling because there are now, metallo-β-lactamases that have the capability of inactivating the two most widely used classes of β-lactam containing antibiotics, penems and cephems (Phelan et al. 2014).

1.5 Overcoming Resistance to β-lactam Antibiotics with Medicinal Chemistry

Traditional medicinal chemistry utilizes knowledge about the biochemical mechanism of resistance to design new therapies for antibiotic resistant bacteria based on previously discovered antibiotic pharmacophores. Structure-activity relationship (SAR) studies for penems revealed that the appendages of the β-lactam pharmacophore could be modified to allow the drug to overcome resistance mechanisms, improve spectrum of activity and pharmacodynamics (Figure 1.1B). This approach is representative of other classes of β-lactams (i.e. cephems and monobactams) (El-Shaboury et al. 2007; Pap-Wallace et al. 2011) (Figure 1.1C) and these efforts have led to vastly improved compounds with extended clinical lifetimes (Powers 2004).
However, all these derivatives share the same pharmacophore (highlighted regions in Figure 1.1A-C), which leaves the possibility that resistance to these drugs could come from the same resistance mechanisms that defeated their predecessors.

Figure 1.1 Medicinal chemistry optimization of different β-lactam antibiotic pharmacophores. Pharmacophores are highlighted with a color: yellow or green = penem, red = cephem, gold = monobactam; A) These are representative structures of β-lactam antibiotics from natural sources; B) Methicillin is the first example of a semi-synthetic derivative of penicillin that was synthesized to decrease affinity for β-lactamase positive S. aureus. In an analogous fashion to methicillin, oxacillin is a semi-synthetic penicillin that has decreased affinity for β-lactamase. The amino-penicillins, ampicillin or amoxicillin, were synthesized to be active in Gram-stain positive and Gram-stain negative bacteria. Piperocillin has reduced susceptibility to β-lactamases produced by Gram-negative bacteria compared to ampicillin and Pivampicillin is a prodrug of ampicillin with improved oral availability; C) These are the structures of selected semi-synthetic cephalosporins, monobactams and carbapenem developed through similar medicinal chemistry efforts as penicillins.
1.6 β-Lactamase inhibitors, the First Resistance Modifying Agents

A complementary strategy to extend the lifetime of antibiotic pharmacophores is to co-administer the drug with a resistance modifying agent (RMA). This technique is known as combination therapy and has been clinically validated since the early 1980s when Augmentin, was approved for clinical use. This groundbreaking therapy utilized the combination of amoxicillin and potassium clavulanate and is the first example of β-lactam antibiotic/ β-lactamase inhibitor combination (White et al. 2004). More than 30 years later it is still a widely used antibiotic treatment. Clavulanate is a natural product that has served as guide to medicinal chemistry efforts to develop synthetic β-lactamase inhibitors (Figure 1.2A). The success of these compounds in the clinic has led to new direct β-lactamase inhibitors that do not rely on the β-lactam pharmacophore to inhibit β-lactamases (Figure 1.2B). There are even inhibitors for metallo-β-lactamases that show promising pre-clinical data (King et al. 2014; Figure 1.2C).

It is encouraging that new, direct β-lactamase inhibitors are being developed, especially those for metallo-β-lactamases; these drugs will become vital medicines to combat antibiotic resistance. Notwithstanding, these inhibitors are not a sustainable solution to antibiotic resistance in MRSA. In order to sustainably address antibiotic resistance, we need a bio-inspired strategy to discover new synthetic antibiotic compounds and RMAs. With this approach we could develop focused screening libraries with excellent 3-dimensional structural diversity. These libraries could then be screened for antibiotic activity or as RMAs for known antibiotics. With unique 3-D structure we anticipate that these libraries will produce lead compounds that have unique
mechanisms of action including regulating antibiotic resistance at the level of genetic expression. This will blossom into new research to discover new drugable biological targets.

**Figure 1.2.** Structures of β-lactamase inhibitors. A) Sulbactam and tazobactam were developed after the discovery of clavulanate and have been used successfully as β-lactamase inhibitors (Draws and Bonomo 2010). The penem core of these β-lactamase inhibitors is similar to penicillin; B) β-lactamase inhibitor without a penem core have been developed, some notable examples are avibactam and relebactam. These compounds are cyclic ureas that were recently approved for clinical use (Toussaint and Gallagher 2015). A different scaffold, RPX7009 is a boronic acid based β-lactamase inhibitor that is currently in clinical trials (Toussaint and Gallagher 2015); C) The natural product, aspergillomarasmine A, is a metallo-β-lactamase inhibitor.

### 1.7 Overview of Dissertation

In the following chapters I will discuss my work towards developing indole alkaloid based antibiotics and resistance modifying agents for β-lactam antibiotics for MRSA. The compounds that will be discussed are inspired by the rich structural diversity of natural product indole alkaloids but none are natural products. They have demonstrated diverse bioactivity reminiscent of natural products. In Chapter 2 I will discuss my use of a previously discovered gold-catalyzed tandem cyclization to perform a structure-activity relationship study for a new class of antibiotic for MRSA, N-benzyl tricyclic indoline alkaloids. I will compare the structure of this antibiotic class with a previously discovered tricyclic indoline alkaloid resistance
modifying agent (RMA) for β-lactam antibiotics. Then in Chapter 3, I will discuss the development of aza-tricyclic indoline RMAs for β-lactam antibiotics in MRSA. In addition I will discuss a novel gold-catalyzed tandem cyclization that was developed to access these RMAs. The new cyclization and synthetic route I describe in Chapter 3 has allowed for gram-scale production of these novel RMAs while also providing RMAs with improved pharmacokinetics ready for pre-clinical evaluation in animal models of infection. The bioinspired strategy that led to the discovery and development of these anti-MRSA compounds could be a sustainable pathway to develop new treatments for MRSA.
CHAPTER 2

DISCOVERY AND INITIAL STRUCTURE-ACTIVITY RELATIONSHIPS OF N-BENZYL TRICYCLIC INDOLINES AS ANTIBACTERIALS FOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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2.1 Introduction

Antibiotic resistance puts a tremendous burden on our healthcare system due to increased morbidity leading to mortality. In addition, drug-resistant infection causes extended hospital stays which greatly increased the cost of care. This continuing problem is being met with improved antibiotic stewardship, diagnostics and tracking of resistant strains. While the aforementioned strategies are necessary to slow the development of major outbreaks of antibiotic resistant bacteria, they will not stop the development of resistance to current antibiotics (Livermore 2003). For this reason we must continuously discover and develop new antibiotics with either novel mechanism of action or that are capable of by-passing known resistance mechanisms. Currently, this demand is not being met on pace with the development of resistance because of the inherent difficulty of antibiotic drug discovery (Harbarth et al 2015).
The history of antibiotic drug-discovery has shown that the majority of antibiotics we use trace their origins to natural sources, and these compounds were likely the low-hanging fruit. With this in mind, high-throughput screening (HTS) has been the prominent method of drug-discovery during the last 20 years. Large pharmaceutical companies have traditionally screened large compound libraries (10,000 to 30,000 distinct chemical entities) against essential biological targets in \textit{in vitro} assays to assess inhibitory activity and locate hit compounds. After identifying a hit, they can screen the potential antibiotics for activity in a whole-cell bacterial growth inhibition assay format to find cell-active leads. This discovery approach has not had success in bringing new drugs to the clinic (Payne et al. 2007). GlaxoSmithKline disclosed that a lack of natural product like structures in their screening library and difficulty in transforming \textit{in vitro} inhibitors to \textit{in vivo} antibiotics were the biggest barriers to their success (Payne et al. 2007).

HTS are also done virtually by performing in silico docking studies using known crystal structures. Using this method, inhibitors of PBP2a have been discovered. These inhibitors were then validated as cell-active antibiotics for MRSA (Bouley et al. 2015, O’Daniel et al. 2014). While this method was successful in this case, it is limited for several reasons. The first and largest reason is that virtual screening requires crystal structures of targets to be available. Secondly, virtual screening can only sample a fraction of drug like chemical space (Irwin and Stoichet, 2005; Polishchuk et al. 2013).

We propose that screening our bio-inspired polycyclic indoline library in a whole-cell inhibition assay could bypass the problems observed in the aforementioned approaches. This library consists of 120 polycyclic indolines with 26 unique core structures (Figure 2.1A). We designed the structural classes of the library to mimic carbocyclic architecture of indole alkaloid natural products (Podoll et al. 2013; Barbour et al. 2014) and this structural diversity will make
up for its relatively small size (120 compounds) compared to commercially available screening libraries (>10,000 compounds). The library was screened against a strain of methicillin-resistant *Staphylococcus aureus* (MRSA) and a methicillin–sensitive *S. aureus* strain (MSSA).

If the antibiotic screen of this library is performed with an antibiotic sensitive *S. aureus* mutant in addition to a multi-antibiotic resistant strain, the hits identified should be more likely to inhibit growth through a novel mechanism of action. A hit was defined as any compound that inhibited growth of MRSA and MSSA at or below the threshold level of 32 µg/mL. After screening our library for antibiotic activity we identified an antibiotic compound, Of4, that shares the same tricyclic indoline core structure as Of1, but has a distinct N-benzyl modification. Of1 was discovered from a previous screening campaign of this library and is a potent resistance modifying agent for β-lactam antibiotics in MRSA. The structures of Of1 and Of4 are compared in Figure 2.1B.

The common tricyclic indoline core shared by Of4 and Of1 is interesting because of the unique activity profiles for these compounds in MRSA. For natural product antibiotics, this seems to be a common theme. For example, clavulenate and penicillin share a similar β-lactam pharmacophore, but one is a resistance modifying agent and the other an antibiotic (Figure 2.1B). Another example is the isonitrile indole alkaloids isolated from cyanobacteria: hapalindole G and ambiguine A isonitrile. The structures of these compounds are compared in Figure 2.1B. These natural products share identical carbocyclic architecture and stereochemistry; however, hapalindole G differs from ambiguine A isonitrile by an alkyl group at C2-position of indole. This difference allows hapalindole G to selectively inhibit the growth of *M. tuberculosis* but not *S. aureus* while ambiguine A isonitrile is selective for *S. aureus* and does not have activity in *M. tuberculosis* (Moore et al, 1987; Mo et al, 2009). We believe that modification of a common
carbocyclic core structure could offer a biomimetic method to generate diverse bioactivity of synthetic anti-MRSA agents. \(N\)-benzyl tricyclic indoline antibiotics share the same tricyclic indoline core as the \(\beta\)-lactam resistance modifying agent, Of1 but have distinct structural modification that give them potent antibiotic activity in multiple MRSA and MSSA strains. In this Chapter, I will describe my initial structure-activity relationship (SAR) study for these antibiotics that has led to an analogue more potent than Of1.

**Figure 2.1** A) A bio-inspired synthetic strategy of a polycyclic indole alkaloid library. B) The shared tricyclic indoline core of antibacterial hit Of4 and resistance modifying agent, Of1 is highlighted in orange; the similar core of the antibiotic penicillin G and resistance modifying agent clavulanic acid is highlighted in green; the shared polycyclic core of bacterial genus selective antibacterial indole alkaloid natural products: hapalindole G and ambiguine A is shown in gold.
2.2 Results and Discussion

Synthesis of Of4 Analogues:

I synthesized analogues 4a-k according to our standard synthetic protocols as described in Scheme 2.1 (Podoll et al, 2013; Yeo et al. 2012; Liu et al. 2011; Chang et al 2014). In brief, alkynyl imines 1a-b were used to prepare indoles 2a-d using our modified one-pot three-component indole synthesis conditions (Yeo et al. 2012). I first activated imines with trifluoroacetic anhydride (TFAA) or benzyl chloroformate (CbzCl), in the presence of 4-dimethylaminopyridine (DMAP). The resulting activated imine was then treated with phenylhydrazine or 4-chlorophenylhydrazine hydrochloride followed by heating for 12 hours with Brønsted acid to give alkynyl indoles 2a-d. Indoles 2a-d were treated with 5 mol% of [bis(trifluoromethanesulfonyl)imidate](triphenylphosphine)gold(I) (2:1) toluene adduct to afford tetracyclic indolines 3a-d through a tandem cyclization pathway previously developed in our lab (Liu et al 2011; Barbour et al. 2014).

With the tetracyclic indolines in hand I was able to perform a one-pot sequential reductive-ring-opening-reductive-amination reaction with substituted benzaldehydes (e.g., 3, 5-dihydroxybenzaldehyde) in the presence of acetic acid and sodium cyanoborohydride to give N-benzyl tricyclic indolines 4a-i and 4k. In addition, I was able to easily and quantitatively cleave the trifluoroacetamide (Tfa) group under basic conditions to give the primary amine analogue 4j (Baker and Castro 1990).
Scheme 2.1. Synthesis of Of4 and analogues 4a-k. See Table 2.1 for description of substituents: R, X, Y and Z. Reagents and conditions: a) TFAA or CbzCl, DMAP, 2 hr, 0-25 °C, then MsOH, 0-25 °C, then ClPhNH-NHNH₂·HCl or PhNHNH₂, 80 °C or 120 °C, 12 hr, DMF, 20-30%; b) 5 mol% [bis(trifluoromethanesulfonyl)imide] (triphenylphosphine)gold(I) (2:1) toluene adduct, 2-12 hr, 50°C, toluene; c) substituted benzaldehyde, NaCNBH₃, AcOH, 2-12 hr, 0-25 °C, MeOH, 30-70 % for two steps; d) Of4, LiOH·H₂O, 24 hr, 25 °C, MeOH, 99%.

Structure-Activity Relationships for Of4:

To determine the biological activity of the analogues I synthesized, I also determined the MIC values for these antibiotics in MSSA using the standard broth microdilution assays, with Of4 and methicillin as controls. The results of these assays are summarized in Table 2.1. Each compound was prepared based on information from the previous compound synthesized. For example, removing the chlorine from the indoline core 4a resulted in a 4-fold loss in activity compared to Of4 (entry 1, Table 2.1).

I initially suspected that the phenolic OH of the N-benzyl group was important to antibiotic activity. I hypothesized that hydrogen bonding capabilities of this group were vital to the antibiotic activity of N-benzyl tricyclic indolines. This guided my efforts to determine the
importance of $N$-benzyl functional group substitution on antibiotic activity, and I started by synthesizing compounds, $4b$-$d$, that have no hydrogen bond donating capabilities on the $N$-benzyl group (entries 3-5, **Table 2.1**). I expected the unsubstituted benzyl analog $4d$ to display decreased activity but I was surprised to find that 3,5-dimethoxy $4b$ and 3,5-difluoro $4c$ to have abolished activity. I found that these compounds did not display antibiotic activity within the range of the experiment.

From these results it followed that synthesizing a compound with additional hydrogen bond donating capability might improve activity. In this vein, I synthesized and tested compound $4e$ and found that the 3,4,5-trihydroxy group led to a loss of antibiotic activity comparable to compounds $4b$-$d$. I attributed this to intramolecular hydrogen bonding.

To test this I prepared analogues with different dihydroxybenzyl substitution patterns such as: *ortho, meta*-substitution $4f$, and *ortho, para*-substitution $4g$ (entries 7 and 8, **Table 2.1**). Surprisingly, these compounds had equivalent activity to Of4. Specifically, the *ortho-meta* analogue could have similar intramolecular hydrogen bonding as $4e$ but it retains activity comparable to Of1. These two analogues suggest that hydrogen bonding might not be the sole reason for loss of activity in $4e$.

I next aimed my study on reducing the side chain length of Of4 by one carbon to prepare analogue $4h$ (entry 9, **Table 2.1**). This compound displayed a promising 2-fold increase in antibiotic activity compared to Of4. Using this information I made an analogue with a shorter side chain but I added a more acidic OH to the benzyl appendage, $4i$ (entry 10, **Table 2.1**). This compound is the same substitution pattern as $4g$ but with a carboxylic acid at the 4 position.
instead of a phenolic OH. I found that activity was decreased in a similar manner as analogues 4b-e, despite the improvement to activity by reducing the side chain length.

Table 2.1. Structures and MIC values of Of4 analogues against MSSA.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Z¹</th>
<th>Z²</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>Y</th>
<th>MICᵃ,ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Of4</td>
<td>Tfa</td>
<td>Cl</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>4a</td>
<td>Tfa</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>&gt;128</td>
</tr>
<tr>
<td>3</td>
<td>4b</td>
<td>Tfa</td>
<td>Cl</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>&gt;128</td>
</tr>
<tr>
<td>4</td>
<td>4c</td>
<td>Tfa</td>
<td>Cl</td>
<td>F</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>&gt;128</td>
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<tr>
<td>5</td>
<td>4d</td>
<td>Tfa</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>&gt;128</td>
</tr>
<tr>
<td>6</td>
<td>4e</td>
<td>Tfa</td>
<td>Cl</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>(CH₂)₂</td>
<td>&gt;128</td>
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<td>7</td>
<td>4f</td>
<td>Tfa</td>
<td>Cl</td>
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</tr>
<tr>
<td>9</td>
<td>4h</td>
<td>Tfa</td>
<td>Cl</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>CH₂</td>
<td>16</td>
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<tr>
<td>10</td>
<td>4i</td>
<td>Tfa</td>
<td>Cl</td>
<td>H</td>
<td>COOH</td>
<td>H</td>
<td>OH</td>
<td>CH₂</td>
<td>&gt;128</td>
</tr>
<tr>
<td>11</td>
<td>4j</td>
<td>H</td>
<td>Cl</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>(CH₂)₂</td>
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<td>Cbz</td>
<td>Cl</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>CH₂</td>
<td>4</td>
</tr>
</tbody>
</table>

ᵃAll MIC values are reported in µg/mL; ᵇMSSA strain ATCC 25923.

I next set out to investigate the role of the Tfa group on the side chain. To achieve this, I removed Tfa under basic conditions to reveal the primary amine, 4j (entry 11, Table 2.1). This compound resulted in a 2-fold loss of activity compared with Of4. However, when I replaced Tfa
with carboxybenzyl (Cbz) to give $4k$ (entry 12, Table 2.1) I found an 8-fold increase in activity compared to Of1. This activity was comparable to the methicillin growth controls and $4k$ became a new lead compound.

**Evaluation of Mammalian Toxicity:**

An important consideration in antibiotic development is minimizing off-target mammalian side effects that could lead to toxicity. For example, the MIC of Of4 is more than three times greater than its half growth inhibition concentration ($\text{GI}_{50}$) in human cervical adenocarcinoma (HeLa) cells. However, with $4k$, we found that the $\text{GI}_{50}$ is double the MIC. The growth inhibition of $4k$ in HeLa cells is shown in Figure 2.2.

![Viability curve](image)

**Figure 2.2.** Viability curve that allows the determination of concentration of compound $4k$ that inhibited the growth of half the population of HeLa cells
Scope of Antibiotic Activity:

With a more potent analogue in hand, I wanted to explore the scope of antibacterial activity of N-benzyl tricyclic indolines in four different representative MRSA strains. I reasoned that if 4k has a novel mechanism of action it should have equivalent activity in multiple MRSA strains with diverse geographical origin, genetic background and resistance profiles. The results are summarized in Table 2.2. I found that analogue 4k did indeed have the same MIC as in MSSA (i.e., 4 µg/mL) for all four MRSA strains tested.

Table 2.2. Evaluation of the MIC values of 4k in a panel of MRSA strains. a

<table>
<thead>
<tr>
<th>Antibacterial</th>
<th>ATCC BAA-44 b</th>
<th>ATCC 33592 c</th>
<th>ATCC BAA-1720 d</th>
<th>NRS 100 e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Of4</td>
<td>32</td>
<td>N.T. f</td>
<td>N.T. f</td>
<td>N.T. f</td>
</tr>
<tr>
<td>4k</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>methicillin</td>
<td>&gt;128</td>
<td>128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

a All MIC values are reported in µg/mL; b An Iberian clone resistant to methicillin, imipenem, cephalothin, erythromycin, ciprofloxacin, doxycycline, clindamycin, rifampin; c A hospital-acquired strain isolated in the United States clone resistant to methicillin and gentamicin; d A hospital-acquired strain, isolated in the United Kingdom, a.k.a. MRSA 252; e A community-acquired strain isolated in the United Kingdom, resistant to methicillin, a.k.a. COL; f Not tested

These strains come from different selection environments and possess known resistance to a wide range of clinically used antibiotics of different structural and mechanistic classes, such as: β-lactams (e.g., amoxicillin/clavulanate, cephalothin, and imipenem), aminoglycosides (e.g. gentamicin), tetracyclines, macrolides (e.g. erythromycin), rifampicins (e.g. rifampin), fluoroquinolones (e.g. ciprofloxacin), and clindamycin. For example, MRSA BAA-44 was originally isolated from a hospital in Portugal (Sanches et al. 1995). MRSA ATCC 33592 is a
hospital-acquired clone from the United States (Schaefler et al. 1981). ATCC BAA-1720 (a.k.a. MRSA 252) is a hospital-acquired strain from the United Kingdom (Holden et al. 2004). NRS 100 (a.k.a. COL) is a community-acquired MRSA strain from the United Kingdom (Tomasz et al. 1989). MRSA 252 is genetically diverse compared to other sequenced MRSA strains, particularly NRS 100.

2.3 Conclusions

We have successfully applied a whole-cell antibiotic screen to our bio-inspired polycyclic indoline library and discovered a novel class of antibiotics for MRSA, N-benzyl tricyclic indolines. Our versatile synthetic route allowed me to efficiently perform a preliminary SAR study to quickly improve the antibiotic activity of screening hit Of4 to give a more potent lead, 4k. This compound demonstrates equivalent activity in MSSA as well as multiple MRSA strains. I believe this could be an indication that 4k acts through a novel antibiotic mechanism that will not be immediately met with resistance from S. aureus. Another important insight I found from this study is that 4k has the same tricyclic indoline core structure as the resistance-modifying agent, Of1. In particular, I found that N-modification of the indoline core (i.e., dihydroxybenzyl) and side chain carbamate (i.e., Cbz) can produce potent antibacterial activity in S. aureus. However, previous SAR studies of Of1 showed that N-modification of the tricyclic indoline and changing the side chain to a carbamate abolished the resistance-modifying activity of Of1 (Chang et al 2014). I believe that modifications of a common structural core can selectively alter biological function in tricyclic indolines and the application of this strategy could be very valuable to develop novel anti-MRSA compounds.
2.4 Outlook

Future SAR studies will need to explore multiple changes to the side chain nitrogen functional group to identify other carbamates, amides or sulfonamides that can replace Cbz without sacrificing activity (Figure 1.2). In addition to this we will need to explore the side-chain length, the indoline halogen substitution pattern and non-aromatic ring size. I suspect that it is not the hydrogen bonding capabilities of the 3,5-dihydroxy group that is key to antibiotic activity, but rather the nucleophilicity of the substituents on the benzyl group. In this light future studies should explore replacing the phenolic OH with more nucleophilic groups such as 3,5-dithio or 3-hydroxy-5-amino. In addition to structural SAR, it would be valuable to synthesize analogues with positive, negative and zwitterionic formal charge states. I think that adding charged groups to this compound will lead to improved water solubility, lower mammalian toxicity and antibiotic activity in Gram-stain negative bacteria.

Figure 2.3. Future SAR study for N-benzyl tricyclic indoline antibiotics

In addition to more in depth SAR, we will need to study the mechanism of action of N-benzyl tricyclic indoline antibiotics. However, this is not a simple task, but generally the strategy is as follows. First we would want to determine if the compound is a bacteriostatic or
bactericidal antibiotic by examining growth curves of *S. aureus* in the presence of different concentrations of antibiotic. This will guide which types of *S. aureus* mutants to obtain from the Network of Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). With appropriate mutants we could look to see if any strains were resistant to 4k. These resistant mutants could then provide insight into mechanism of action to that would allow us to design more advanced experiments like isotopic labeling, pull-down studies or target mutational studies.

This will narrow our suspected biological targets and allow us to isolate biomolecules of interest. With purified biological targets we can characterize biochemical parameters, and obtain crystal structures if not already available. If the biological target is in fact novel, we can use the information about mechanism of action to drive a more in depth medicinal chemistry study based on rational design to find new antibiotics for this hypothetical target.

### 2.5 Experimental Methods

**Chemical Synthesis:**

Unless otherwise noted, reagents were obtained commercially and used without further purification. Dichloromethane (DCM) was purchased from Fisher Chemical and distilled from CaH₂ under a nitrogen atmosphere prior to use. Toluene (Tol) was purchased from Sigma-Aldrich and distilled from CaH₂ under a nitrogen atmosphere prior to use. Triethylamine (TEA) and methanol (MeOH) were purchased from Fisher Chemical. Anhydrous carbon tetrachloride (CCl₄), acetonitrile (ACN), *N,N*-dimethylformamide (DMF) and 1,4-dioxane (Dioxane) were purchased from Sigma-Aldrich. Ethyl acetate (EtOAc) was purchased from Macron Fine Chemicals. Chloroform (CHCl₃) and hexanes (Hex) were purchased from EMD Chemicals.
EtOAc, Hex, MeOH, CHCl₃ and TEA were used as elution solvents for thin-layer chromatography (TLC) and flash column chromatography. TLC analysis was performed on Silicycle SiliaPlate® F-254 TLC plates. Flash column chromatography was carried out on Silicycle SiliaFlash® P60 silica gel. ¹H, ¹³C NMR spectra were recorded with Bruker Avance-III 300 spectrometer, Varian INOVA 400 NMR or Varian INOVA 500 spectrometer and referenced to Chloroform-d (¹H NMR: 7.26 ppm; ¹³C NMR: 77.16 ppm), Acetone-d₆ (¹H NMR: 2.05 ppm; ¹³C NMR: 29.84, 206.26 ppm) or Methanol-d₄ (¹H NMR: 4.87, 3.31; ¹³C NMR: 49.0). Mass spectral and analytical data were obtained via the Agilent 6120 LC/MS.

**Synthesis of 2d:**

Benzyl chloroformate (0.379 g, 2.22 mmol, 1.2 equiv.) was added to a solution of 4-dimethylaminopyridine (0.271 g, 2.22 mmol, 1.2 equiv.) in anhydrous DMF (1.2 M) at 0 °C. The reaction was stirred at 23 °C for 30 minutes. A solution of the alkynyl imine 1b (0.300 g, 2.22 mmol, 1.0 equiv.) in anhydrous DMF (1.2 M) was added and the reaction was stirred at 23 °C for another 30 minutes followed by dropwise addition of methanesulfonic acid (0.360 ml, 5.55 mmol, 3.0 equiv.) at 0 °C. The reaction was then stirred at 23 °C for 2 hours. 4-Chlorophenylhydrazine hydrochloride (0.497 g, 2.77 mmol, 1.5 equiv) was added and the mixture was stirred for 1 hour at 23 °C. The reaction was diluted to 0.1 M with anhydrous DMF and heated to 120 °C for 12 hours in a sealed tube. After 12 hours the reaction was cooled to room temperature and diluted with ethyl acetate then washed with saturated aqueous NaHCO₃ then saturated aqueous NaCl. The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to
give benzyl-2-(5-chloro-2-(pent-4-ynyl)-1H-indol-3-yl) ethylcarbamate (2d): TLC (hexanes:ethyl acetate 3:1 v/v) $R_f = 0.25$; light yellow oil, 20 % yield, $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ 7.94 (bs, 1H), 7.49 (s, 1H), 7.36 (s, 5H), 7.21 (d, $J = 8.5$ Hz, 1H), 7.10 (dd, $J = 8.5$, 2.0 Hz, 1H), 5.12 (s, 2H), 4.79 (s, 1H), 3.45 (q, $J = 6.7$ Hz, 2H), 2.91 (t, $J = 6.9$ Hz, 2H), 2.87 (t, $J = 7.4$ Hz, 2H), 2.26 – 2.16 (m, 2H), 2.08 (t, 1H), 1.85 (p, $J = 8.3$, 7.8 Hz, 2H).

**Synthesis of 3d:**

A solution of indole 2d (30 mg, 0.076 mmol, 1.0 equiv.) in anhydrous toluene (0.10 M) was added to a suspension of [bis(trifluoromethanesulfonyl)imide] (triphenylphosphine) gold(I) (2:1) toluene adduct (5.97 mg, 3.80 µmol, 0.05 equiv.) in anhydrous toluene (0.10 M). The solution was heated to 50 °C under argon atmosphere until TLC showed that no starting material remained (12 h). The reaction mixture was then filtered through a short pad of silica gel. The filtrate was concentrated *in vacuo* and used directly in the next step.

**Synthesis of 4k:**

To a solution of indoline 3d (0.015 g, 0.038 mmol, 1.0 equiv.) in anhydrous methanol (0.20 M) was added acetic acid (4.34 µl, 0.076 mmol, 2.0 equiv.), and sodium cyanoborohydride (9.55 mg, 0.152 mmol, 4.0 equiv.) at 0 °C. The reaction was stirred for 30 minutes at which point 3, 5-dihydroxybenzaldehyde (7.87 mg, 0.057 mmol, 1.5 equiv.) was added and the reaction was warmed to room temperature overnight. The solvent was removed *in vacuo* to give a residue, which was dissolved in ethyl acetate, and washed with saturated aqueous NaHCO$_3$ then saturated aqueous NaCl. The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated *in vacuo* to give a crude product, which was purified by column chromatography on
silica gel (gradient 10:1 to 2:1 hexanes: ethyl acetate) to give benzyl-2-(6-chloro-9-(3,5-
dihydroxybenzyl) -4-methylene-2,3,4,4a,9,9a-hexahydro- 1H-carbazol-4a-yl)ethylcarbamate
(4k): TLC (hexanes : ethyl acetate 2:1) Rf = 0.20; yellow residue, 50% yield; \( ^1 \)H NMR (500 MHz, Chloroform-\( d \)) \( \delta \) 7.42 – 7.29 (m, 5H), 7.03 (dd, \( J = 8.3, 2.2 \) Hz, 1H), 6.84 (d, \( J = 2.2 \) Hz, 1H), 6.64 (d, \( J = 2.2 \) Hz, 2H), 6.47 (s, 2H), 6.39 – 6.26 (m, 2H), 5.13 (s, 1H), 5.12 – 5.04 (m, 2H), 4.96 (d, \( J = 8.9 \) Hz, 2H), 4.45 (d, \( J = 15.5 \) Hz, 1H), 3.97 (d, \( J = 15.5 \) Hz, 1H), 3.69 (dd, \( J = 9.1, 5.6 \) Hz, 1H), 3.41 m, 1H), 3.24 (m, 1H), 2.33 – 2.21 (m, 2H), 2.03 – 1.93 (m, 1H), 1.79 (m, 2H), 1.65 (s, 1H), 1.57 (m, 1H), 1.32 – 1.19 (m, 1H), 1.12 (m, 1H); \( ^{13} \)C NMR (75 MHz, CDCl3) \( \delta \) 157.83, 156.86, 147.91, 146.57, 140.27, 136.01, 135.80, 128.59, 128.32, 128.17, 127.82, 122.87, 121.97, 111.52, 107.40, 106.56, 101.81, 67.31, 66.33, 51.25, 47.16, 37.75, 36.56, 33.19, 29.71, 25.36, 21.07; MS (m/z): calcd. for C\(_{30}\)H\(_{31}\)ClN\(_2\)O\(_4\), 518.2; found [M+H]\(^+\), 519.2.

**General Biology:**

* S. aureus* strains ATCC BAA-44 and ATCC 25923 were generously donated by Daniel Feldheim and Charles McHenry (Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO), respectively. * S. aureus* strains ATCC 33592 and BAA-1720 were purchased from the ATCC (www.ATCC.org). * S. aureus* strain NRS 100 (COL) was acquired free of charge from the Network on Antimicrobial Resistance in * S. aureus* (NARSA, http://www.narsa.net/). Mueller Hinton Broth (MHB) was used as growth media for all MIC experiments. MHB was purchased from HIMEDIA through VWR (cat: 95039-356).
HeLa cells were used for the mammalian cytotoxicity experiments. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin.

MRSA strain ATCC BAA-44 was a gift from the laboratory of Daniel Feldheim. Strains NRS100, NRS384, and NR-46414 were obtained from BEI Resources (https://www.beiresources.org/). HeLa cells were purchased from ATCC (www.atcc.org). CellTiter-Glo® luminescent cell viability assay kit was purchased from Promega Corp.

**Polycyclic Indoline Alkaloid Library Antibacterial Screen:**

The indoline compounds were initially assayed for their ability to inhibit growth of *S. aureus* (ATCC 25923) using the BacTiter-Glo™ Microbial Cell Viability Assay according to the manufacturer’s instructions (www.promega.com). Briefly, white, flat-bottom, 384-well plates (Corning 3570) were prepared by the addition of 35 µl of MHB using a MicroFlo Select liquid dispenser (BioTek). Compounds from a 4 mM DMSO solution 96-well source plate were then pinned into each well of the 384-well assay plate using the CyBi® -Well 96-channel simultaneous pippetor (CyBio). Next, an overnight culture of MSSA ATCC 25923 was diluted 25,000-fold and 5 µl of this dilution was added to each well of the 384-well plate. The final concentration of each compound used in the screen was 20 µM, and the final dilution of bacteria inoculated was 1:100,000. Compounds were screened in quadruplicate. Plates were incubated at 37°C with shaking for 5 hours before the BacTiter-Glo™ Microbial Cell Viability Assay was performed and the signal recorded in an Envision Multilabel Plate Reader (Perkin Elmer).
Minimum inhibitory Concentration (MIC) Assay:

Compounds were evaluated for their MICs in different *S. aureus* strains using the CLSI standard microdilution method.\(^1\) Briefly, compounds were prepared in 96-well microplates (USA Scientific CytoOne 96-well TC plate, cat: CC7682-7596) at twice the intended final concentration and two-fold serial dilutions were prepared down the columns of the plate to afford a suitable concentration range. The inoculum was prepared by diluting a bacterial day culture (OD\(_{600}\) 0.30-0.40) to OD\(_{600}\) 0.002. This dilution was then added to 96-well microplates for a final inoculum concentration of OD\(_{600}\) 0.001. All plates were incubated at 37°C with shaking overnight (18 hours) and results were interpreted by visual inspection. The MIC was defined as the lowest concentration at which no observable growth was present.

Mammalian Cell Viability Assay:

To evaluate the cytotoxicity of 4k in mammalian cells, a viability assay was carried out using the CellTiter-Glo™ luminescent cell viability assay (www.promega.com). HeLa cells were seeded on white, cell-culture treated 96-well plates (Corning: 3917) with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. The initial cell density was 8,000 cells/well. The medium volume for each well was 100 µL. Cells were incubated at 37 °C in 5% CO\(_2\)/95% air for 16 hours. The medium was removed from each well and replaced with 99 µL of warmed fresh medium. To each well, 1 µL of 4k was added in DMSO to afford a final concentration of 0.4-50 µM. Each concentration was performed in triplicate. After incubation at 37 °C for another 24 hours, the
plates were equilibrated to room temperature for 10 minutes. 40 μL of CellTiter-Glo™ reagent (Promega) was added to each well and mixed for 30 minutes on an orbital shaker to stabilize the luminescent signal. The luminescence of each sample was recorded by an Envision Multilabel Plate Reader (Perkin Elmer).
CHAPTER 3

PROPERTY-GUIDED SYNTHESIS OF AZA-TRICYCLIC INDOLINES:
DEVELOPMENT OF GOLD CATALYSIS EN ROUTE


3.1 Introduction

As a society, we require the ability to manage infection. Antibiotic resistance is threatening this ability and progressing faster than we can develop new treatments. On average, it takes 10-15 years to bring a new antibiotic candidate to the clinic, but less than one year for resistance to develop (Payne et al. 2007; Clatworthy et al. 2007). At our current pace we can expect to return to an era where surgical rooms, burn units and cancer treatment centers are plagued with untreatable microorganisms. New technology has allowed researchers to uncover a novel natural product based antibiotic with promising preclinical activity (Ling et al. 2015). In addition, virtual screening has discovered two potential antibiotics for MRSA (Bouley et al. 2015, O’Daniel et al. 2014). Undoubtedly, these discoveries are positive developments; however, they are only a piece of the puzzle. Resistance is inevitable and these new antibiotics will eventually
select for resistant strains. In order to continue our ability to control bacterial infection we must expand our thinking about how we approach developing new therapies for bacterial infection.

One approach is to use resistance modifying agents (RMAs) to extend the clinical lifetime of previously discovered antibiotics (Kalan and Wright 2011; Miller et al 2001; White et al. 2004; Worthington and Melander 2013). β-lactamase inhibitors have been used in combination with β-lactam antibiotics for more than 30 years (Figure 3.1A). In fact, these inhibitors are the only RMAs approved by the Food and Drug Administration (FDA). However, directly inhibiting β-lactamases is not the only way to modify antibiotic resistance in bacteria and is by no means the most efficient method. An alternative strategy emerging in our group and others suggests that RMAs can modify resistance without directly inhibiting β-lactamase (Podoll et al. 2013; Boudreau et al. 2015; Harris et al. 2012)

Figure 3.1. A) Examples of direct β-lactamase inhibitors; B) Structures of RMAs that can resensitize MRSA to β-lactam antibiotics through a unique mechanism by regulating the signaling that controls β-lactam specific resistance.
In our group, we discovered a tricyclic indoline that can restore β-lactam antibiotic activity in MRSA (Podoll et al. 2013). Through a follow-up SAR study, we discovered that altering the halogenation pattern of Of1 gave a more potent analogue, 1, as shown in Figure 3.1A (Chang et al. 2014). We also found that a tetracyclic indolenine, reminiscent of Of1, was able to resensitize MRSA to β-lactam antibiotics (Xu et al. 2015). Mechanism of action studies demonstrated that Of1 decreases the expression of PBP2a and β-lactamase genes in MRSA but it does not directly inhibit the enzymatic activity of β-lactamase in a nitrocefin competition assay (Podoll 2015).

We believe that Of1 acts through the the two most common mechanisms of resistance to β-lactam antibiotics in MRSA: β-lactamases and PBP2a. These mechanisms of resistance are inducible based on environmental signals (Zhang et al. 2001) and controlled by analogous mechanisms of induction. The accepted mechanism for the induction of these resistance mechanisms is presented in Figure 3.2A-C. In the case of Of1, the BlaI transcription repressor is of interest. Our current evidence suggests that Of1 targets this protein and stabilizes the dimeric form bound to the transcriptional start site of the bla operon. We believe that this allows Of1 to block the ability of MRSA to detect β-lactam antibiotics which in turn keeps active β-lactamases and PBP2a from being produced (Podoll 2015).

This mechanism of action is unique compared to other RMAs (e.g. clavulante, avibactam) and inspired us to advance this compound to pre-clinical testing. We quickly discovered that Of1 and 1 are not suitable for animal studies. I attribute this to their poor physiochemical properties (i.e. poor water solubility and pharmacokinetics). In support of this we found several cheminformatic studies that highlighted the bias of antibacterial agents toward more hydrophillic chemical space (O’Shea and Moser 2008; Brown et al. 2014; Camp et al. 2015). These studies
quantify hydrophillicity by determining the average calculated logarithm of distribution coefficient at pH 7.4 ($c\log D_{7.4}$). This value predicts the distribution of an organic compound in octanol or aqueous buffer at a specific pH and lower $c\log D_{7.4}$ values are characteristic of hydrophillic compounds.

Figure 3.2. The BlaR/I signaling pathway in MRSA from Boudreau et al. 2015 and Borbulyevch et al. 2011; A) BlaR is a membrane bound protein that has an extracellular sensory domain and an intracellular zinc protease. In the absence of β-lactam antibiotic, BlaR is inactive ($I$). When β-lactam antibiotic is present, BlaR becomes activated ($O$). BlaR is phosphorylated once activated and in the $O$ state, BlaR destroys the transcriptional repressor BlaI via its intracellular zinc protease; B) BlaI controls the expression of the bla operon. BlaI binds the transcriptional start site of the operon as a dimer, and this binding keeps the expression of blaI, blaR and blaZ at basal levels and as BlaI is destroyed, transcription levels of these three genes increases. This results in increased amounts of functional β-lactamase (part C). The BlaR/I system is analogous in function to the PBP2a regulatory system, MecR/I and BlaR/I is capable of regulating both β-lactamase and PBP2a production in MRSA (Baba et al. 2002).
O’Shea and Moser calculated that the average clogD$_{7.4}$ of marketed agents targeting Gram-positive bacteria (e.g., *S. aureus*) is -0.2. Most clinical β-lactam antibiotics (e.g., amoxicillin and cefazoline) and β-lactamase inhibitors (e.g., clavulanate) contain ionizable groups, which make these compounds extremely hydrophilic. I found the clogD$_{7.4}$ for Of1 (i.e., 4.8) to be drastically higher than typical antibacterial agents. From this observation we set out to better understand how we can leverage physical properties to develop RMAs like Of1 into leads that can enter into pre-clinical studies.

In our previous SAR study for Of1 we found a more potent analogue 1, but this compound had comparable clogD$_{7.4}$ as Of1 (Chang et al. 2014). While this study did not improve physiochemical properties, it did provide some important guidelines. First, it told us that we cannot modify the indole nitrogen or sulfonamide without drastically sacrificing biological activity. But we could make different halogen substitutions on the sulfonamide and indoline position. This study informed us that our current synthetic strategy would not allow us to drastically decrease clogD$_{7.4}$ while maintaining biological activity.

One region that had not yet been modified in Of1 was the c-ring (*Figure 3.3A*). Through a virtual SAR study we found that incorporating nitrogen into this ring, 2, drastically decreases the cLogD$_{7.4}$ (*Figure 3.3A*). The tricyclic indoline core structure would then be transformed into a piperidine-fused indoline or aza-tricyclic indoline (ATI). ATIs could then be further modified at the c-ring nitrogen to further decrease the cLogD$_{7.4}$. In addition, the ATI is a common motif in many biologically active indole alkaloid natural products (e.g., ajmaline and reserpine) as shown in *Figure 3.3B*. These findings prompted our investigation into using ATIs as an analogous form of Of1. We planned to synthesize ATIs by adapting our previously approach for tricyclic indolines (Podoll et al. 2013).
As shown in Figure 3.3C, tricyclic indolines were previously prepared from a gold-catalyzed tandem cyclization of alkynyl indoles 5 followed by a ring-opening reduction of the resulting tetracyclic indolines 6. We envisioned that a similar gold- or platinum-catalyzed cyclization of a propargyl amine-conjugated tryptamine derivative 8 could be used to form the aza-tetracyclic indolines 9, which may undergo a ring-opening reduction to produce the desired ATIs 2 (Figure 3.3D). These ATIs could then be modified to introduce functional groups that could improve pharmacokinetics and physical properties without sacrificing anti-MRSA activity.

In this Chapter, I will discuss the property-guided synthesis of ATIs. This work necessitated the development of a novel and highly efficient gold-catalyzed tandem cyclization to synthesize ATIs. I will also describe my rationale for introducing ionized groups to the ATI core. Lastly we will conclude with the results of the biological evaluation of ATIs. Through this study we were able to produce a novel resistance modifying agent for β-lactam antibiotics that has vastly improved physical and biological properties compared to the parent compound, Of1.
Figure 3.3. Design and proposed targeted synthesis of aza-tricyclic indolines. (A) Structures of tricyclic indolines and aza-tricyclic indolines. (B) Structures of aza-tricyclic indoline-containing natural products. (C) Previously reported synthetic route to Of1 using a diversity synthesis strategy. (D) Planned targeted synthetic route for aza-tricyclic indolines. TMB: 3,4,5-trimethoxybenzoyl.
1.2 Results and Discussion

**Synthesis of Indole Alkyne Cyclization Precursors:**

To synthesize the cyclization precursor $8$, I first employed our one-pot, three-component reaction using imine $3$ to give 2-methyl tryptamines, both nitrogens of which were protected with *tert*-butoxycarbonyl (Boc) groups to afford $7a$ as described in **Scheme 3.1**.

**Scheme 3.1** Synthesis of the cyclization precursor $8a$. Reagents and conditions: (a) ClsCl, DMAP, 3 then 4-bromo-2-fluorophenylhydrazine•HCl, CH$_3$SO$_3$H, CH$_3$CN, 80°C, 59 %; (b) Boc$_2$O, DMAP, Et$_3$N, CH$_3$Cl$_2$, 23°C, 86 %; (c) NBS, BPO, CCl$_4$, 85 °C; (d) H$_2$NCH$_2$CCH, CH$_3$CN, -10 °C 83 % in two steps; (e) TFA, CH$_2$Cl$_2$, 0 °C; (f) NsCl, Et$_3$N, CH$_2$Cl$_2$, 89% in two steps. ClsCl, 4-chlorobenzenesulfonyl chloride; DMAP, 4-dimethyl-aminopyridine; Boc, *tert*-butoxycarbonyl; NBS, N-bromo succinimide; BPO, benzoyl peroxide; NsCl, 4-nitrobenzenesulfonyl chloride.

I then selectively brominated the methyl group of $7a$ through a radical bromination to produce bromide $10a$; this reaction provides one product in quantitative yields. I then used $10a$ to alkylate propargyl amine through a $S$_n2 process. I found that if I performed the reaction with an
excess of propargyl amine at -10 °C, the reaction stopped at the secondary amine, but with one equivalent of propargyl amine at room temperature, the tertiary amine was the major product. I deprotected both Boc groups using trifluoroacetic acid (TFA) then I protected the secondary amine nitrogen with 4-nitrobenzenesulfonyl (Ns) group to give the cyclization precursor 8a. The Ns group was chosen because it has been widely utilized in transition metal-catalyzed cyclization of propargyl amine derivatives and it can be removed under mild conditions for further functionalization of the nitrogen atom (Hashmi et al. 2000; Ferrer and Echavarren 2006; Ferrer et al. 2006; Dong et al. 2013; Xu et al. 2015; Miller et al. 2014).

As opposed to the previously developed diversity synthesis strategy, this synthetic route is more efficient and produces only one regioisomer of the alkynyl indole cyclization precursor (Figure 3.2C & D). The bromination reaction was also found to be highly selective and can tolerate substrates containing a carboxybenzyl group on the tryptamine nitrogen. Thus, a number of cyclization precursors with a variety of different substitutions were efficiently synthesized in good yield using this route.

**Development of a Tandem Cyclization of Propargylamine-Conjugated Tryptamine Derivatives:**

With cyclization precursor 8a in hand, we set out to develop a tandem cyclization reaction to give the desired tetracyclic indoline 9a (Table 3.1). Platinum/gold catalysis has proved well suited for tandem cyclizations of alkynyl indoles (Hashmi and Rudolph 2008; Rudolph and Hashmi 2012; Pflasterer and Hashmi 2015; Barbour et al. 2014; Fensterbank and Malacria 2014); in our previous work, we found triphenylphosphinegold(I) bis(trifluoromethane-
sulfonfonylimidate (Ph₃PAuNTf₂) to be an efficient catalyst for the tandem cyclization of alkynyl indoles 5 (Liu et al 2011; Podoll et al. 2013; Chang et al. 2014; Barbour et al. 2014). We first evaluated this catalyst in our initial attempt to cyclize 8a. 8a appeared to be much less reactive than our previous substrates 5 (Figure 3.1C) and the desired product 9a was formed in low yield shown in entry 1 of Table 3.1.

The conversion of this reaction was improved by increasing the temperature, but the yield remained sub-optimal (entry 2). We hypothesized that the low reactivity of 8a was due to its lower electron density and greater steric hindrance of the alkyne, compared to the alkyne in 5. Therefore, we evaluated catalysts with higher electrophilicity (Obradors and Echavarren 2014), as well as platinum catalysts, which have been utilized in efficient carbocyclizations of 1,6-enynes (Gruit et al. 2011; Tsukano et al. 2012).

In our system, the majority of the substrate decomposed with all platinum catalysts tested (entries 3-5). Returning to gold catalysis, we found that cationic gold-carbene complex gave a small amount of product (entry 7), and its chloride salt was incapable of promoting the reaction. IPr is an N-heterocyclic carbene ligand and can stabilize Au-Au bonding. This type of bonding could occur on intermediates involved in gold-catalyzed transformations and may lead to undesired reaction pathways, resulting in low yield of the desired product (Obradors and Echavarren 2014; Espada et al. 2015; Muñiz et al. 2011). Hence, we decided to screen additional phosphine ligands.

On the observation that ligands like IPr gave poor yields, we hypothesized that increasing the bulk of the phosphine ligand may minimize the formation of di-gold complexes that could lead to side reactions and poor catalyst turnover.
Table 3.1 Optimization of the tandem cyclization reaction.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>T [°C]</th>
<th>Yield [%][a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph3PAuNTf2</td>
<td>Toluene</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>Ph3PAuNTf2</td>
<td>Toluene</td>
<td>90</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>PtCl2</td>
<td>Toluene</td>
<td>90</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td>PtCl4</td>
<td>Toluene</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>H2PtCl6•6H2O</td>
<td>Toluene</td>
<td>90</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6</td>
<td>IPrAuCl</td>
<td>Toluene</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>IPrAuCl/AgSbF6</td>
<td>Toluene</td>
<td>90</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>XPhosAuNTf2</td>
<td>Toluene</td>
<td>90</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>XPhosAuNTf2</td>
<td>1,4-Dioxane</td>
<td>90</td>
<td>97</td>
</tr>
</tbody>
</table>

[a] Yields were calculated based on ¹H NMR integration using 4-dimethylaminopyridine as an internal standard.

Considering the lower electron density of the alkyne in 8a, we evaluated a gold catalyst with electron-rich phosphine ligands. Gratifyingly, we found that catalyst 2-dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl gold(I) bis(trifluoromethanesulfonyl)imide
(XPhosAuNTf₂) gave a much improved yield over Ph₃PAuNTf₂ (entry 8). Further survey of additional solvents (e.g., dichloroethane, dioxane, and nitromethane) in this reaction identified 1,4-dioxane as optimal, and a near quantitative yield of the tandem cyclization product 9a was obtained (entry 9).

I have proposed a plausible mechanism for the formation of 9a from 8a in Scheme 2, based on our previous understanding of this type of gold-catalyzed tandem cyclizations (Liu et al. 2010; Noey et al. 2011; Barbour et al. 2014). The catalyst will first coordinate with the alkyne of substrate 8a to form intermediate I. The 3-position of indole will then attack the activated alkyne in a 6-exo-dig mode to give iminium ion II. A secondary cyclization will afford III that can be deprotonated to give intermediate IV, which is followed by proto-deauration to yield the desired product 9a.

Scheme 3.2 Proposed mechanism for tandem cyclization. Ns= 4-nitrobenzenesulfonamide; Cls= 4-chlorobenzenesulfonamide
Evaluation of the Scope and Limitations of the Optimized Tandem Cyclization Reaction:

After cyclization conditions were optimized, I synthesized nine additional cyclization precursors using the synthetic approach described in Scheme 3.1 to give compounds 8b-j. The substrate scope is provided in Table 3.2. These compounds allowed me to explore the scope of the new cyclization reaction conditions to assess the feasibility of using this reaction to perform structure-activity relationship (SAR) studies for ATIs. I found that substrates bearing a variety of functional groups on the propargyl amine nitrogen atom were tolerated under the optimized cyclization conditions. Trifluoroacetamide 8b provided a lower, but acceptable yield of the cyclization product (entry 2). Methanesulfonamide and two other sulfonamides all gave excellent yields (entries 3-5); however, higher catalyst loading was required to consume the 4-chloro-2-nitro-benzensulfonamide 8d in 12 hours. Additionally, benzyloxycarbonyl (Cbz; entry 6) was also tolerated on the secondary amine with a yield similar to the amide substrate 8f, probably due to the formation of some undesired oxazole byproduct through a gold-catalyzed cyclodebenzylation process (Weyrauch et al. 2010).

Our previous SAR studies on Of1 showed that halogen substitutions on indoline can significantly impact their biological activity (Chang et al. 2014). This inspired me to evaluate substrates with different halogenation patterns on the indole. The results showed that indole with different halogen substitutions in the acyclic precursors were well tolerated and all gave excellent yields. I discovered that removing fluorine from the aromatic system led to a faster reaction (entry 7), which I attribute to increased nucleophilicity of the indole. I tested substrates with the Cbz group on the tryptamine side chain because Cbz can be removed in the presence of Ns, to allow for side chain modification of ATIs later on. Substrates with a Cbz group in this
position appeared to be more reactive than their sulfonamide counterparts and gave excellent yields, regardless of the substitutions on the indole (entries 8-10). With Cbz I could decrease temperature and reaction time to give excellent yields. The Cbz protected nitrogen appears to be more nucleophilic than the sulfonamide protected nitrogen; I attribute this to the sulfonamide nitrogen interacting with the gold catalyst to form a more stable complex than the carbamate nitrogen.
Table 3.2 Scope of gold-catalyzed tandem cyclization.[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8a</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>2</td>
<td>8b</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>3</td>
<td>8c</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>4</td>
<td>8d</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>5</td>
<td>8e</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>6</td>
<td>8f</td>
<td>Br</td>
<td>F</td>
<td>Cls</td>
</tr>
<tr>
<td>7</td>
<td>8g</td>
<td>Br</td>
<td>H</td>
<td>Cls</td>
</tr>
<tr>
<td>8</td>
<td>8h</td>
<td>Cl</td>
<td>H</td>
<td>Cbz</td>
</tr>
<tr>
<td>9</td>
<td>8i</td>
<td>Cl</td>
<td>Cl</td>
<td>Cbz</td>
</tr>
<tr>
<td>10</td>
<td>8j</td>
<td>F</td>
<td>H</td>
<td>Cbz</td>
</tr>
</tbody>
</table>

[a] Standard reaction conditions (5 mol % XPhosAuNTf₂, 1,4-dioxane, 90 °C, 12 hours unless noted otherwise.  
b] Isolated yield based on complete conversion of substrate.  
c] 10 mol % catalyst used.  
d] 2 hour reaction time.  
e] 2 hour reaction time at 60 °C. Cls, 4-chloro-benzenesulfonyl; Ns, 4-nitro-benzenesulfonyl; Tfa, trifluoroacetyl; Ms, methanesulfonyl; CNs, 4-chloro-2-nitro-benzenesulfonyl; Ts, 4-methyl-benzenesulfonyl; Cbz, benzyloxy carbonyl.
Conversion of Cyclization Products to ATIs:

To explore ATIs with unique physical properties, I converted the cyclization products, 9a-c, to ATIs under slightly modified reductive ring-opening conditions to furnish 11a-c (Scheme 3.3).

Scheme 3.3 Synthesis of ATIs. Reagents and conditions: (a) TFA, NaCNBH3, THF/MeOH, 0°C; (b) PhSH, K2CO3, CH3CN, 60 °C; (c) i. 15, CH2Cl2, 23 °C; ii. TFA, CH2Cl2, 0 °C; (d) i. tert-butyl bromoacetate, Et3N, CH2Cl2, 0 °C; ii. TFA, CH2Cl2, 0 °C. Tfa, trifluoroacetamide; TFA, trifluoroacetic acid; Cls, 4-chloro-benzenesulfonyl.

To synthesize additional ATIs with even lower clogD7.4 values, I removed the Ns group of 11a under nucleophilic conditions to afford secondary amine 12. I then functionalized the secondary amine as a cationic guanidine group 13 using a commercially available guanidinylation reagent 15 followed by deprotection with trifluoroacetic acid. I also synthesized a zwitterion analogue 14 that was also synthesized by treating 12 with tert-butyl bromoacetate followed by deprotection with trifluoroacetic acid.
I hypothesized that introducing ionizable groups to the ATI core would do several beneficial things for ATIs: 1) decrease off-target mammalian toxicity, 2) improve metabolic stability and 3) improve RMA activity in MRSA. These predictions come from two pieces of information, the first being that the cellular membrane of animals and bacteria alike generally have membranes that are negatively charged, however Gram-positive bacteria have a thick cell-wall consisting of a net of peptidoglycan interlaced with additional anionic techoic acid polymers not present in mammalian cells (Silhavy et al. 2010). These charged polymers could potentially sequester ATIs on the bacterial cell-wall and increase their effective concentration to \textit{S. aureus}. Ionizing RMAs would also decrease their general permeability to mammalian cells and force ATI distribution in a mammalian system to be controlled by specific transporters in mammalian cells (Shitara et al 2006).

In addition to controlling distribution in mammalian cells, adding charge to ATIs could also decrease general drug-metabolism. Cytochrome P450 (CYP) enzymes are iron dependent metalloenzymes involved in drug degradation (Guenguerich 2007). It has been suggested that hyrdophobic compounds have greater affinity for these enzymes (Lewis et al. 2004). I believe that ionized ATIs will have decreased affinity for the CYP enzyme active site. This will effectively increase the concentration of the drug in a mammalian system.

**Comparison of the Biological Activity of Of1 and ATIs:**

ATIs **11-14** represent a wide range of clogD values, and we evaluated their minimum re-sensitizing concentrations (MRCs) for cefazolin (i.e., a first-generation cephalosporin) and amoxicillin/clavulanic acid (i.e., Augmentin, amox/clav) as well as their minimum inhibitory
concentrations (MICs) in a multi-drug resistant MRSA strain, ATCC BAA-44. The results of our biological evaluations are given in Table 3.3. Any compounds with promising MRC and MIC were then evaluated for potential mammalian toxicity by determining their half growth inhibitory concentrations (GI\textsubscript{50}) in human cervical adenocarcinoma (HeLa) cells.

**Table 3.3** Comparison of the biological activity of Of1 and ATIs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>clogD\textsubscript{7.4}</th>
<th>MRC Cef\textsuperscript{[a]}</th>
<th>MRC Amox/clav\textsuperscript{[a]}</th>
<th>MIC\textsuperscript{[a]}</th>
<th>GI\textsubscript{50} \textsuperscript{[a, b]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Of1</td>
<td>4.8</td>
<td>4</td>
<td>4</td>
<td>&gt;128</td>
<td>17</td>
</tr>
<tr>
<td>11a</td>
<td>4.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>NT</td>
</tr>
<tr>
<td>11b</td>
<td>4.1</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>NT</td>
</tr>
<tr>
<td>11c</td>
<td>2.5</td>
<td>4</td>
<td>4</td>
<td>&gt;32</td>
<td>19</td>
</tr>
<tr>
<td>12</td>
<td>2.0</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td>13</td>
<td>0.74</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>0.54</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>NT</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} All MIC, MRC, and GI\textsubscript{50} values are in µg mL\textsuperscript{-1}; \textsuperscript{[b]} Determined for HeLa cells. Cef-cefazolin; amox/clav- amoxicillin/clavulanic acid; NT- Not tested.

We found that the two sulfonamides, 11a and 11b, showed no antibacterial activity or potentiating effects for cefazolin (cef) or amox/clav. Interestingly, the trifluoroacetamide analogue 11c showed similar activity profile as Of1; it potentiates the activity of β-lactams in BAA-44 without observable antibacterial activity. The more polar analogue, amine 12, exhibited potent antibacterial activity on its own, albeit with significant mammalian toxicity. More interestingly, the guanidine analogue 13 showed not only improved β-lactam-potentiating
activity (MRCs = 2 µg mL\(^{-1}\)) for both cefazolin and amox/clav) with moderate antibacterial activity (MIC = 8 µg mL\(^{-1}\)), but also much lower mammalian toxicity with the GI\(_{50}\) of 40 µg mL\(^{-1}\) in HeLa cells. Although compound 14 is zwitterionic under physiological conditions and has even lower clogD value, it showed no anti-MRSA activity. Further evaluations of 13 in a variety of MRSA strains, such as community-acquired MRSA strains NRS-100 and NRS-384, and vancomycin-resistant S. aureus (VRSA) strain NR-46414, also gave the same results (MRCs = 2 µg mL\(^{-1}\)).

**Evaluation of the Physiochemical and Pharmacokinetic Properties of ATI 13**:

We next evaluated the physiochemical properties of ATI 13 and found that this compound had vastly improved physical properties compared to Of1. To begin, we set out to compare their aqueous solubility, we found the saturation concentration of Of1 to be 0.86 µg mL\(^{-1}\), lower than its effective MRC in MRSA. However, the saturated concentration of ATI 13 was much higher at 587 µg mL\(^{-1}\), almost 300 fold its MRC in MRSA.\(^{[23]}\) These findings led us to pursue *in vivo* evaluation of pharmacokinetic (PK) properties of 13. A single dose (30 mg kg\(^{-1}\)) of 13, administered to mice through intraperitoneal (IP) injection was well tolerated with only minor clinical observations and 13 showed promising in vivo PK properties (**Figure 3.3**).

The half-life (T\(_{1/2}\)) of 13 was 2.5 hr and a maximum concentration (C\(_{\text{max}}\)) of 8.0 µg mL\(^{-1}\) was reached quickly after 30 minutes. The area under the curve (AUC) to the last data point at 24 hr was 18.2 hr µg mL\(^{-1}\), which matched the AUC calculated to infinity. We predicted that, similar to highly polar β-lactam antibiotics, 13 should have low general membrane permeability. To test this, permeability assessments were performed using MDCK cells. The permeability of
13 was comparable to mannitol, which is a low permeability marker (i.e., 2.11E-06±3.53E-07 cm s$^{-1}$ for 13; 1.51E-06 ± 2.67E-07 cm s$^{-1}$ for mannitol). For comparison, the permeability of metoprolol, a high permeability marker, was measured as 5.35E-05±6.05E-06 cm s$^{-1}$ in this assay. Despite low permeability in vitro, in vivo PK studies through IP delivery shows that 13 can reach plasma levels at approximately 4xMRC in MRSA (Figure 3.2). Overall, ATI 13 exhibited high aqueous solubility, low mammalian membrane permeability and good PK properties in vivo.

Figure 3.4. Single-dose Pharmacodynamics study of 13 in mice. Each data point represents the mean and SD of n = 3 mice. The dashed horizontal line is the MRC of 13 in cell culture.
3.3 Conclusions

We developed an efficient synthetic route to allow the facile synthesis of a series of tetracyclic indolines that could then be modified into aza-tricyclic indolines (ATIs). Using this route I was able to prepare ATIs that possess improved physiochemical properties, compared to the parent compound Of1; several of these ATIs had intriguing anti-MRSA activity. In particular, I synthesized a guanidine-containing ATI, 13, that showed not only improved anti-MRSA activity and lower mammalian toxicity, but also significantly improved aqueous solubility and metabolic stability. The improvement of these properties allowed further studies of this novel class of resistance-modifying agents in mice. The *in vivo* pharmacokinetic data of 13 are highly promising and reminiscent of some β-lactam antibiotics, suggesting them as suitable companions for β-lactam antibiotics.

To synthesize the multiple tetracyclic indoline precursors for ATIs, I needed to develop a novel and efficient tandem cyclization. We discovered that we could use a commercially available gold(I) catalyst to afford tetracyclic indolines in good to excellent yields. This reaction proved to be tolerant of a variety of different substrates and is well suited for a future structure-activity relationship study of 13. This work further demonstrates that gold catalysis is especially well suited to synthesize complex polycyclic heterocycles with applicability in a medicinal chemistry context. This is a new and exciting direction, which builds upon the proven utility of gold catalysis as a highly versatile strategy to develop complex carbocyclic ring systems. More specifically, gold cycloisomerisations of alkynyl indoles provide selective and robust reactions that can be used to develop not only diverse and complex compound collection for the discovery
of novel chemical probes, but also drug-like compounds for further evaluation of efficacy in in vivo models. In this context, future directions towards asymmetric versions of cycloisomerisations, and recyclable catalysts could blossom the utility of gold catalysis in medicinal chemistry and chemical biology.

### 3.4 Outlook

ATI 13 is a valuable lead compound that will greatly advance our ability to progress ATIs to pre-clinical studies. The structure-activity relationships of this compound are not yet fully elucidated. Our future directions will be to perform several in-depth SAR studies to determine the pharmacophore of ATI 13 and also explore other modifications of ATIs that could provide chemical probes to study the mechanism of action for these compounds. In particular, we wish to see if the sulfonamide present in Of1 and ATIs is necessary for biological activity. We know that in the case of Of1 this group is important, but it is unclear in ATIs. With this in mind we plan to synthesize a variety of ATI analogues of 11c and 13 with different substitution on the side chain nitrogen and indoline halogen substitution. We also plan to synthesize enantiomerically enriched 13 by utilizing asymmetric ligands in out tandem cyclization. The results of these future studies will allow us to then determine if 13 can function in an animal model of infection.
3.5 Experimental Methods

**General Biology Information:**

The detailed procedures for the MIC assay, MRC assay, GI50 determination, physiochemical assays (i.e. water solubility, membrane permeability), Phase I metabolic assay and single-dose pharmacokinetic studies are presented in the appendix.

**General Chemical Synthesis:**

Unless otherwise noted, reagents were obtained commercially and used without further purification. Dichloromethane (DCM) was purchased from Fisher Chemical and distilled from CaH₂ under a nitrogen atmosphere prior to use. Toluene (Tol) was purchased from Sigma-Aldrich and distilled from CaH₂ under a nitrogen atmosphere prior to use. Triethylamine (TEA) and methanol (MeOH) were purchased from Fisher Chemical. Anhydrous carbon tetrachloride (CCl₄), acetonitrile (ACN), N,N-dimethylformamide (DMF) and 1,4-dioxane (Dioxane) were purchased from Sigma-Aldrich. Ethyl acetate (EtOAc) was purchased from Macron Fine Chemicals. Chloroform (CHCl₃) and hexanes (Hex) were purchased from EMD Chemicals. EtOAc, Hex, MeOH, CHCl₃ and TEA were used as elution solvents for thin-layer chromatography (TLC) and flash column chromatography. TLC analysis was performed on Silicycle SiliaPlate® F-254 TLC plates. Flash column chromatography was carried out on Silicycle SiliaFlash® P60 silica gel. ¹H, ¹³C NMR spectra were recorded with Bruker Avance-III 300 spectrometer, Varian INOVA 400 NMR or Varian INOVA 500 spectrometer and referenced
to Chloroform-d (1H NMR: 7.26 ppm; 13C NMR: 77.16 ppm), Acetone-d₆ (1H NMR: 2.05 ppm; 13C NMR: 29.84, 206.26 ppm) or Methanol-d₄ (1H NMR: 4.87, 3.31; 13C NMR: 49.0). Mass spectral and analytical data were obtained via the Waters Synapt G2 HDMS quadrupole/ToF, electrospray ionization (ESI) operated by the Central Analytical Laboratory, University of Colorado Boulder. Infrared (IR) spectra were recorded with an Agilent Corey FT-IR spectrometer. Melting point (m.p.) determinations were performed on Digimelt melting point apparatus (Stanford Research Systems) and are uncorrected.

**Preparation of Cyclization Precursors (8a-j) and Analytical Data:**

**General Procedure A (Indole Synthesis Reaction):**

4-Chloro-benzenesulfonyl chloride (6.33 g, 30 mmol) was added to a solution of 4-dimethylaminopyridine (DMAP) (3.67 g, 30 mmol) in anhydrous DMF (25 mL) at 0 °C. The reaction was stirred at 23° C for 30 minutes. A solution of 2-methyl-1-pyrroline 3 (2.08 g, 25 mmol) in anhydrous DMF (25 mL) was added and the reaction was stirred at the same temperature for 1 hour. Methanesulfonic acid (4.87 mL, 75 mmol) was added to the reaction at 0 °C. The reaction was then stirred at 23° C for 2 hours. 4-Bromo-2-fluoro-phenyl hydrazine hydrochloride (9.06 g, 35.7 mmol) was added and stirred for an additional hour at 23° C. The
reaction was then heated to 85 °C for 12 hours in a sealed tube. The reaction was then cooled to room temperature and concentrated in vacuo. The resulting residue was dissolved in ethyl acetate and washed with saturated aqueous solution of NaHCO₃ followed by brine. The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel eluting with 70:30 Hex: EtOAc to give S1 as an off-white solid (59.7% yield, 6.65 g, 14.9 mmol).

(S1)

**Melting point:** 163.5 – 165.3 °C

**IR (thin film):** 3324, 3260, 2925, 2877, 1646, 1573, 1480, 1413, 1398, 1313 cm⁻¹;

**¹H NMR** (400 MHz, Acetone-­d₆) δ 10.47 (s, 1H), 7.75 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 7.33 (d, J = 1.5 Hz, 1H), 6.94 (dd, J = 10.5, 1.5 Hz, 1H), 6.65 (t, J = 6.3 Hz, 1H), 3.16 (q, J = 6.8 Hz, 2H), 2.86 (t, J = 6.8 Hz, 2H), 2.37 (s, 3H) ppm;

**¹³C NMR** (101 MHz, Acetone-­d₆) δ 149.35 (d, J = 246.7 Hz), 140.81, 138.57, 136.59, 134.32 (d, J = 6.6 Hz), 129.88, 129.29, 122.94 (d, J = 12.8 Hz), 117.08 (d, J = 3.3 Hz), 110.83 (d, J = 8.1 Hz), 109.43 (d, J = 20.4 Hz), 109.24 (d, J = 2.2 Hz), 44.27, 25.44, 11.48 ppm;

**HRMS (m/z):** [M+Li]⁺ calcd. for C₁₇H₁₅BrClFN₂O₂SLi, 449.9861; found, 449.9861.

**S2-S5 Prepared Using General Procedure A**

(S2) was obtained as a white solid (80.3%, 0.343 g, 0.803 mmol).
Melting point: 133.2 – 134.6 °C;

IR (thin film): 3350, 3264, 2925, 2873, 1573, 1477, 1410, 1350 cm\(^{-1}\);

\(^1\)H NMR (400 MHz, Chloroform-\(d\)) δ 7.95 (s, 1H), 7.62 (d, \(J = 8.6\) Hz, 2H), 7.41 – 7.32 (m, 3H), 7.18 (dd, \(J = 8.6, 1.9\) Hz, 1H), 7.12 (d, \(J = 8.5\) Hz, 1H), 4.45 (t, \(J = 6.2\) Hz, 1H), 3.21 (q, \(J = 6.5\) Hz, 2H), 2.84 (t, \(J = 6.5\) Hz, 2H), 2.34 (s, 3H) ppm;

\(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) δ 139.19, 134.16, 133.98, 129.88, 129.43, 128.40, 124.23, 120.18, 112.86, 112.00, 106.77, 43.05, 24.59, 11.88 ppm;

HRMS (m/z): [M+Li]\(^+\) calcd. for C\(_{17}\)H\(_{16}\)BrClN\(_2\)O\(_2\)Li, 431.9956; found, 431.9958.

(S3) was obtained as a yellow oil (44.6%, 0.660 g, 1.92 mmol).

IR (thin film): 3417, 3327, 2936, 1696,1590, 1518, 1473, 1458, 1240 cm\(^{-1}\);

\(^1\)H NMR (400 MHz, Chloroform-\(d\)) δ 8.24 (s, 1H), 7.47 (d, \(J = 2.0\) Hz, 1H), 7.40 – 7.33 (m, 5H), 7.13 (d, \(J = 8.5\) Hz, 1H), 7.07 (dd, \(J = 8.5, 2.0\) Hz, 1H), 5.13 (s, 2H), 4.95 (t, \(J = 6.1\) Hz, 1H), 3.40 (q, \(J = 6.6\) Hz, 2H), 2.86 (t, \(J = 6.6\) Hz, 2H), 2.28 (s, 3H) ppm;

\(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) δ 156.55, 136.53, 133.89, 133.64, 129.67, 128.56, 128.16, 128.09, 124.85, 121.10, 117.21, 111.39, 108.01, 66.67, 41.44, 24.43, 11.51 ppm;

HRMS (m/z): [M+Li]\(^+\) calcd. for C\(_{19}\)H\(_{19}\)ClN\(_2\)O\(_2\)Li, 348.1286; found, 348.1288.
(S4) was obtained as a yellow oil (35.8%, 0.430 g, 1.14 mmol).

**IR (thin film):** 3424, 3316, 3033, 3070, 2929, 1696, 1518, 1480, 1458, 1443, 1238 cm⁻¹;

**¹H NMR** (400 MHz, Chloroform-d) δ 8.12 (s, 1H), 7.40 – 7.29 (m, 6H), 7.11 (d, J = 1.8 Hz, 1H), 5.10 (s, 2H), 5.00 (s, 1H), 3.39 (q, J = 6.6 Hz, 2H), 2.85 (t, J = 6.6 Hz, 2H), 2.35 (s, 3H) ppm;

**¹³C NMR** (101 MHz, Chloroform-d) δ 156.48, 136.59, 134.65, 131.10, 130.52, 128.64, 128.56, 128.26, 128.08, 125.14, 120.63, 116.29, 116.22, 109.62, 66.80, 41.41, 24.71, 11.75 ppm;

**HRMS (m/z):** [M+Na]+ calcd. for C₁₉H₁₈Cl₂N₂O₂Na, 399.0643; found, 399.0655.

(S5) was obtained as a clear oil (55% 0.540 g, 1.68 mmol).

**IR (thin film):** 3417, 3324, 3037, 2925, 1696, 1588, 1518, 1488, 1458, 1235 cm⁻¹;

**¹H NMR** (400 MHz, Chloroform-d) δ 7.82 (s, 1H), 7.40 – 7.29 (m, 5H), 7.20 – 7.09 (m, 2H), 6.85 (td, J = 9.1, 2.5 Hz, 1H), 5.10 (bs, 2H), 4.78 (bs, 1H), 3.42 (q, J = 6.7 Hz, 2H), 2.87 (t, J = 6.7 Hz, 2H), 2.33 (s, 3H) ppm;

**¹³C NMR** (101 MHz, Chloroform-d) δ 157.96 (d, J = 234.3 Hz), 156.52, 138.60, 134.17, 131.77, 128.66, 128.26, 110.88 (d, J = 9.7 Hz), 109.30 (d, J = 26.0 Hz), 108.79, 103.05 (d, J = 23.3 Hz), 66.75, 41.42, 24.69, 11.84 ppm;

**HRMS (m/z):** [M+Na]+ calcd. for C₁₉H₁₉FN₂O₂Na, 349.1328; found, 349.1335.
General Procedure B (N,N’-diBoc Protection):

To a solution of S1 (2.75 g, 6.16 mmol, 12.3 mL, 0.50 M in DCM), DMAP (1.50 g, 12.3 mmol), TEA (1.71 mL, 12.3 mmol) and di-tert-butyl dicarbonate (Boc₂O) (3.36 g, 15.4 mmol) were added. The reaction was stirred at room temperature for 48 hours then washed with water and brine. The organic layer was then dried over Na₂SO₄ concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel eluting with 90:10 Hex:EtOAc to give 7a as a yellow oil (86%, 3.42 g, 5.30 mmol).

(7a).

IR (thin film): 2981, 2936, 1734, 1581, 1462, 1354, 1320, 1257 cm⁻¹;

¹H NMR (400 MHz, Chloroform-δ) δ 7.77 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 1.7 Hz, 1H), 7.45 (d, J = 8.7 Hz, 2H), 7.09 (dd, J = 11.6, 1.7 Hz, 1H), 3.94 – 3.87 (m, 2H), 3.09 – 3.02 (m, 2H), 2.52 (s, 3H), 1.63 (s, 9H), 1.40 (s, 9H) ppm;

¹³C NMR (101 MHz, Chloroform-δ) δ 150.63, 149.60, 149.36 (d, J = 255.2 Hz), 139.97, 138.48, 137.28, 134.57 (d, J = 4.3 Hz), 129.43, 129.04, 121.59 (d, J = 8.9 Hz), 116.69 (d, J = 3.7 Hz), 115.01 (d, J = 8.8 Hz), 113.80 (d, J = 25.2 Hz), 113.11 (d, J = 1.9 Hz), 85.06, 84.73, 46.77, 28.07, 27.95, 25.14, 12.87 ppm;

HRMS (m/z): [M+K]⁺ calcd. for C₂₇H₃₁BrClF₇N₂O₆SK, 683.0396; found, 683.0396.

7g-i Prepared Using General Procedure B.
(7g) was obtained as a white solid (62%, 0.182 g, 0.290 mmol).

**Melting point:** 101.2 – 102.7 °C

**IR (thin film):** 2981, 2936, 1730, 1588, 1462, 1354, 1279, 1261 cm⁻¹;

**1H NMR** (400 MHz, Chloroform-\(d\)) \(\delta\) 7.98 (d, \(J = 8.8\) Hz, 1H), 7.77 (d, \(J = 8.7\) Hz, 2H), 7.66 (d, \(J = 2.0\) Hz, 1H), 7.43 (d, \(J = 8.7\) Hz, 2H), 7.32 (dd, \(J = 8.8, 2.0\) Hz, 1H), 3.97 – 3.82 (m, 2H), 3.13 – 3.03 (m, 2H), 2.57 (s, 3H), 1.68 (s, 9H), 1.40 (s, 9H) ppm;

**13C NMR** (101 MHz, Chloroform-\(d\)) \(\delta\) 150.80, 150.43, 139.91, 138.53, 136.02, 134.56, 131.98, 131.63, 129.45, 129.01, 126.39, 120.51, 117.03, 116.15, 113.86, 85.01, 84.31, 46.79, 28.39, 28.09, 25.02, 14.15 ppm;

**HRMS (m/z):** [M+K]⁺ calcd. for C\(_{27}\)H\(_{32}\)BrClN\(_2\)O\(_6\)S, 665.0490; found, 665.0482.

(7h) was obtained as a yellow oil (78%, 0.420 g, 0.773 mmol).

**IR (thin film):** 2981, 2929, 1789, 1726, 1598, 1462, 1357, 1294, 1261 cm⁻¹;
1H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 8.01 (d, \(J = 8.8\) Hz, 1H), 7.43 (d, \(J = 2.1\) Hz, 1H), 7.41 – 7.32 (m, 5H), 7.17 (dd, \(J = 8.8, 2.1\) Hz, 1H), 5.21 (s, 2H), 3.85 – 3.69 (m, 2H), 2.97 – 2.79 (m, 2H), 2.42 (s, 3H), 1.66 (s, 9H), 1.43 (s, 9H) ppm;

13C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 153.76, 151.90, 150.44, 135.94, 135.48, 134.19, 131.28, 128.73, 128.65, 128.59, 128.29, 123.54, 117.39, 116.59, 114.33, 84.10, 83.10, 68.58, 46.08, 28.37, 28.03, 23.55, 13.96 ppm;


(7i) was obtained as a yellow oil (60%, 0.345 g, 0.598 mmol).

IR (thin film): 2981, 2936, 1798, 1748, 1592, 1562, 1458, 1339, 1249 cm\(^{-1}\);

1H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 7.41 – 7.26 (m, 6H), 7.17 (d, \(J = 1.8\) Hz, 1H), 5.15 (s, 2H), 3.92 – 3.59 (m, 2H), 2.92 – 2.70 (m, 2H), 2.31 (s, 3H), 1.62 (s, 9H), 1.42 (s, 9H) ppm;

13C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 153.48, 151.73, 149.40, 136.83, 135.22, 133.20, 130.68, 128.52, 128.41, 128.39, 127.68, 124.05, 119.49, 116.21, 112.29, 84.83, 82.87, 77.48, 77.16, 76.84, 68.38, 45.94, 27.85, 27.71, 27.67, 23.40, 11.87 ppm;

HRMS (m/z): [M+Li]+ calcd. for C\(_{29}\)H\(_{34}\)Cl\(_2\)N\(_2\)O\(_6\)Li, 582.1945; found, 582.1949.
(7j) as a yellow oil (86%, 0.423 g, 0.804 mmol).

**IR (thin film):** 2985, 2933, 1786, 1722, 1607, 1569, 1369, 1279, 1167 cm⁻¹;

**¹H NMR** (400 MHz, Chloroform-d) δ 8.02 (dd, J = 9.1, 4.6 Hz, 1H), 7.43 – 7.31 (m, 5H), 7.09 (dd, J = 8.8, 2.6 Hz, 1H), 6.93 (td, J = 9.1, 2.6 Hz, 1H), 5.21 (s, 2H), 3.82 – 3.64 (m, 2H), 2.90 – 2.79 (m, 2H), 2.43 (s, 3H), 1.66 (s, 9H), 1.43 (s, 9H) ppm;

**¹³C NMR** (101 MHz, Chloroform-d) δ 159.40 (d, J = 238.9 Hz), 153.77, 151.96, 150.54, 136.15, 135.46, 132.06, 131.01 (d, J = 9.3 Hz), 128.75, 128.65, 128.63, 116.47 (d, J = 9.0 Hz), 114.72 (d, J = 3.8 Hz), 110.94 (d, J = 24.6 Hz), 103.45 (d, J = 23.5 Hz), 83.91, 83.08, 68.62, 46.04, 28.39, 28.02, 23.65, 14.05 ppm;

**HRMS (m/z):** [M+Li]⁺ calcd. for C₂₉H₃₅FN₂O₆Li, 532.2631; found, 532.2639.

**General Procedure C (Bromination of 7a):**

N-Bromosuccinimide (NBS) (0.276 g, 1.54 mmol) and benzoyl peroxide (BPO) (0.050 g, 0.155 mmol) were added to a solution of 7a (1.00 g, 1.54 mmol, 6.2 mL, 0.25 M in CCl₄) followed by heating at 85 °C for 1 hour. Insoluble material was removed by filtering the crude
reaction through celite, followed by concentration *in vacuo* to give 10a, which was used directly in the next step without further purification.

**General Procedure D (Alkylation of Propargyl Amine):**

A solution of crude 10a (1.12 g, 1.54 mmol, 7.7 mL, 0.20 M in ACN) was added dropwise to a solution of propargyl amine (0.986 mL, 15.4 mmol, 10 M in ACN) at -10 °C over the course of 10 minutes. After addition of indole was complete, the reaction was stirred for 1 hour in the ice bath. The reaction was diluted 20x with EtOAc and washed with saturated bicarbonate solution followed by washing with brine. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give a crude product, which was purified by column chromatography on silica gel eluting with 50:48:2 Hex:EtOAc:TEA to give S6 as a clear oil (83%, 0.893 g, 1.278 mmol). Yield was calculated based on 8a. (S6).

**IR (thin film):** 3316, 2981, 2936, 1734, 1577, 1462, 1357, 1324, 1261 cm⁻¹;
\(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 7.79 (d, \(J = 8.6\) Hz, 2H), 7.55 (d, \(J = 1.6\) Hz, 1H), 7.45 (d, \(J = 8.6\) Hz, 2H), 7.13 (dd, \(J = 11.5, 1.6\) Hz, 1H), 4.10 (s, 2H), 3.99 – 3.91 (m, 2H), 3.45 (d, \(J = 2.4\) Hz, 2H), 3.21 – 3.11 (m, 2H), 2.21 (t, \(J = 2.4\) Hz, 1H), 1.63 (s, 9H), 1.40 (s, 9H) ppm;

\(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 150.72, 149.72, 149.63 (d, \(J = 255.6\) Hz), 139.99, 138.50, 138.13, 134.04 (d, \(J = 4.1\) Hz), 129.46, 129.07, 122.16 (d, \(J = 9.2\) Hz), 117.53 (d, \(J = 3.7\) Hz), 115.51 (d, \(J = 1.9\) Hz), 115.03 (d, \(J = 8.5\) Hz), 114.54 (d, \(J = 24.9\) Hz), 85.15, 85.13, 82.11, 71.81, 47.18, 42.52, 37.49, 28.09, 27.76, 25.22 ppm;

HRMS (m/z): [M+H]\(^+\) calcd. for C\(_{30}\)H\(_{34}\)BrClF\(_3\)N\(_3\)O\(_6\)S, 698.1102; found, 698.1116.

S12-15 Prepared Using General Procedure C then D.

(S7) was obtained as a clear oil (60%, 0.241 g, 0.354 mmol).

IR (thin film): 3294, 2929, 2873, 1726, 1480, 1458, 1398, 1354, 1320, 1279 cm\(^{-1}\);
**1H NMR** (400 MHz, Chloroform-*)δ* 7.89 (d, *J* = 8.9 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 1.9 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.35 (dd, *J* = 8.9, 1.9 Hz, 1H), 4.15 (s, 2H), 3.98 – 3.86 (m, 2H), 3.46 (d, *J* = 2.5 Hz, 2H), 3.23 – 3.13 (m, 2H), 2.19 (t, *J* = 2.5 Hz, 1H), 1.69 (s, 9H), 1.41 (s, 9H) ppm;

**13C NMR** (101 MHz, Chloroform-*)δ* 150.65, 150.13, 139.84, 138.46, 136.74, 134.56, 131.13, 129.37, 128.98, 127.29, 121.31, 117.25, 116.83, 116.21, 85.00, 84.89, 82.23, 71.62, 47.09, 43.24, 37.34, 28.29, 28.03, 25.08 ppm;

**HRMS (m/z):** [M+H]⁺ calcd. for C₃₀H₃₆BrClN₃O₆S, 680.1197; found, 680.1204.

(S8) was obtained as a clear oil (53%, 0.290 g, 0.456 mmol).

**IR (thin film):** 3301, 2981, 2933, 1726, 1507, 1458, 1372, 1354, 1324 cm⁻¹;

**1H NMR** (400 MHz, Chloroform-*)δ* 7.93 (d, *J* = 8.9 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.43 – 7.31 (m, 5H), 7.21 (dd, *J* = 8.9, 2.1 Hz, 1H), 5.23 (s, 2H), 4.07 (s, 2H), 3.84 – 3.75 (m, 2H), 3.35 (d, *J* = 2.4 Hz, 2H), 3.06 – 2.94 (m, 2H), 2.13 (t, *J* = 2.4 Hz, 1H), 1.68 (s, 9H), 1.45 (s, 9H);

**13C NMR** (101 MHz, Chloroform-*)δ* 153.77, 151.93, 150.24, 136.56, 135.52, 134.32, 130.88, 128.76, 128.60, 128.50, 124.58, 118.33, 117.57, 116.91, 84.80, 83.24, 82.37, 71.48, 68.63, 46.41, 43.12, 37.15, 28.37, 28.09, 23.67 ppm;

**HRMS (m/z):** [M+H]⁺ calcd. for C₃₂H₃₉ClN₃O₆, 596.2527; found, 596.2509.
(S9) was obtained as a yellow oil (72%, 0.182 g, 0.289 mmol).

IR (thin film): 3305, 2981, 2929, 1748, 1693, 1454, 1372, 1339, 1249 cm⁻¹;

¹H NMR (400 MHz, Chloroform-d) δ 7.43 (d, J = 1.9 Hz, 1H), 7.37 (d, J = 3.0 Hz, 5H), 7.25 (d, J = 1.9 Hz, 1H), 5.18 (s, 2H), 3.99 (s, 2H), 3.86 – 3.73 (m, 2H), 3.37 (d, J = 2.4 Hz, 2H), 2.99 (dd, J = 8.6, 6.3 Hz, 2H), 2.19 (t, J = 2.4 Hz, 1H), 1.62 (s, 8H), 1.42 (s, 9H) ppm;

¹³C NMR (101 MHz, Chloroform-d) δ 153.76, 151.98, 149.88, 135.41, 132.80, 128.75, 128.61, 128.57, 128.01, 125.34, 120.08, 117.30, 85.81, 83.31, 72.32, 68.66, 46.45, 41.95, 37.12, 28.05, 27.79, 23.62 ppm;

HRMS (m/z): [M+H]+ calcd. for C₃₂H₃₇Cl₂N₃O₆, 630.2137; found, 630.2142.

(S10) was obtained as a clear oil (55%, 0.100 g, 0.173 mmol).

IR (thin film): 3305, 2981, 2933, 1726, 1696, 1622, 1477, 1458, 1372, 1328 cm⁻¹;
\[ ^1\text{H NMR} \ (400 \text{ MHz, Chloroform}-d) \delta 10.19 \ (s, 1\text{H}), 8.50 - 8.38 \ (m, 2\text{H}), 8.22 \ (d, J = 8.8 \text{ Hz, 2H}), 7.39 \ (dd, J = 8.8, 4.5 \text{ Hz, 1H}), 7.36 - 7.25 \ (m, 6\text{H}), 6.92 \ (td, J = 9.2, 2.5 \text{ Hz, 1H}), 6.45 \ (t, J = 6.3 \text{ Hz, 1H}), 5.04 \ (s, 2\text{H}), 4.69 \ (s, 2\text{H}), 4.10 \ (d, J = 2.5 \text{ Hz, 2H}), 3.37 \ (q, J = 6.8 \text{ Hz, 2H}), 2.96 \ (t, J = 7.3 \text{ Hz, 2H}), 2.66 \ (d, J = 2.5 \text{ Hz, 1H}) \text{ ppm}; \]

\[ ^{13}\text{C NMR} \ (101 \text{ MHz, Chloroform}-d) \delta 159.35 \ (d, J = 239.6 \text{ Hz}), 153.76, 151.98, 150.32, 136.72, 135.47, 132.20, 130.60 \ (d, J = 9.4 \text{ Hz}), 128.76, 128.62, 128.59, 117.96 \ (d, J = 3.9 \text{ Hz}), 116.83 \ (d, J = 8.8 \text{ Hz}), 112.09 \ (d, J = 24.7 \text{ Hz}), 104.31 \ (d, J = 23.6 \text{ Hz}), 84.60, 83.21, 82.38, 71.46, 68.66, 46.33, 43.17, 37.11, 28.37, 28.06, 23.74 \text{ ppm}; \]

HRMS (m/z): [M+H]^+ calcd. for C_{32}H_{39}FN_{3}O_{6}, 580.2823; found, 580.2794.

\[ \text{General Procedure E (Boc Deprotection)}: \]

\[ S6 \ (0.293 \text{ g, 0.419 mmol}) \text{ was dissolved in 10 mL 1:1 TFA:DCM then stirred at 0}^\circ \text{C for 2 hours. The solvent was then concentrated in vacuo to give a residue. The residue was diluted with EtOAc and washed with a saturated aqueous solution of NaHCO}_3. \text{ The aqueous was then extracted 3x with EtOAc and the combined organic layers were washed with brine and dried over anhydrous Na}_2\text{SO}_4, \text{ filtered and concentrated in vacuo to give S11 propargyl indole which was used for the next step without further purification.} \]
DCM was shaken in a reparator funnel with H₂O before use in reaction.

**General Procedure F (Protection of Propargyl Amine):**

To a solution of S11 (0.209 g, 0.419 mmol, 1.7 mL, 0.25 M in DCM) was added TEA (0.175 ml, 1.26 mmol), 4-nitro-benzensulfonyl chloride (NsCl) (0.098 g, 0.440 mmol) at 0 °C and warmed to room temperature. The reaction was stirred for 2 hours. The reaction was concentrated then diluted with EtOAc and washed with saturated aqueous solution of NaHCO₃. The organic layer was washed with water, brine and then separated and dried over anhydrous Na₂SO₄. Solids were removed via filtration and then solvent was removed *in vacuo* to give a crude product, which was purified by column chromatography on silica gel eluting with 70:30 Hex:EtOAc to give 8a as a beige solid (89%, 0.255 g, 0.373 mmol).

(8a).

**Melting point:** 169.1 – 170.0 °C;

**IR (thin film):** 3283, 3100, 2925, 2858, 1577, 1536, 1354, 1328 cm⁻¹;

**¹H NMR (400 MHz, Acetone-\(\text{d}_6\))** \(\delta\) 10.85 (s, 1H), 8.44 (d, \(J = 8.8\) Hz, 2H), 8.22 (d, \(J = 8.8\) Hz, 2H), 7.77 (d, \(J = 8.6\) Hz, 2H), 7.53 (d, \(J = 8.6\) Hz, 2H), 7.47 (d, \(J = 1.6\) Hz, 1H), 7.06 (dd, \(J =
10.4, 1.6 Hz, 1H), 6.70 (t, J = 6.2 Hz, 1H), 4.73 (s, 2H), 4.10 (d, J = 2.5 Hz, 2H), 3.20 (t, J = 6.7 Hz, 2H), 2.99 (t, J = 7.0 Hz, 2H), 2.63 (t, J = 2.5 Hz, 1H) ppm;

$^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 151.30, 149.74 (d, J = 248.7 Hz), 144.88, 140.70, 138.66, 133.63 (d, J = 6.1 Hz), 132.43, 130.32, 129.94, 129.29, 125.10, 124.15 (d, J = 13.3 Hz), 118.21 (d, J = 3.5 Hz), 113.51 (d, J = 2.2 Hz), 111.37 (d, J = 7.9 Hz), 111.13 (d, J = 20.2 Hz), 76.84, 76.38, 44.31, 43.00, 37.28, 25.36 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{26}$H$_{21}$BrClFN$_4$O$_6$S$_2$Li, 687.9910; found, 687.9897.

**Alternative Procedure F’ (Protection of Propargyl Amine):**

TFAOSu (0.021 g, 0.100 mmol) was added to a solution of S11 (0.050 g, 0.100 mmol, 0.400 mL, 0.25 M in DCM) at 0 °C and warmed to room temperature. The reaction was stirred for 2 hours. The reaction was quenched by addition of a saturated aqueous solution of NaHCO$_3$ and then diluted with EtOAc. The organic layers were washed with water followed by brine. The organic layer was then dried over anhydrous Na$_2$SO$_4$. Solids were removed via filtration and then solvent was removed *in vacuo* to give a crude product, which was purified by column chromatography on silica gel eluting with 70:30 Hex:EtOAc to give 8b as a clear oil (50%, 0.050 g, 0.050 mmol).
IR (thin film): 3320, 3301, 3268, 2929, 2854, 1696, 1577, 1486, 1428, 1385 cm$^{-1}$;

$^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.72 (s, 1H), 7.82 – 7.72 (m, 4H), 7.55 – 7.48 (m, 6H), 7.08 (ddd, $J = 10.5$, 3.2, 1.6 Hz, 2H), 6.81 – 6.67 (m, 2H), 4.98 (s, 2H), 4.27 (d, $J = 2.4$ Hz, 2H), 3.26 (dt, $J = 11.6$, 4.2 Hz, 4H), 3.07 (t, $J = 7.0$ Hz, 4H), 2.99 (t, $J = 2.5$ Hz, 1H) ppm;

$^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 156.53, 148.84 (d, $J = 247.8$ Hz), 139.85, 137.79, 132.58 (d, $J = 5.8$ Hz), 131.68, 131.23, 129.06, 128.42, 123.25 (d, $J = 13.2$ Hz), 117.44 (d, $J = 3.7$ Hz), 112.94 (d, $J = 1.9$ Hz), 112.43, 110.52 (d, $J = 8.0$ Hz), 110.39, 110.19, 76.88, 74.49, 43.48, 41.72, 36.30, 24.52 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{22}$H$_{17}$BrClF$_4$N$_3$O$_3$SLi, 598.9950; found, 598.9955.

General Procedures E then F were used to Prepare 8c-j.

(8c) was obtained as a white solid (50%, 0.044 g, 0.076 mmol).

Melting point: 185.3 – 186.7 °C;

IR (thin film): 3301, 3320, 3249, 2925, 2854, 1573, 1480, 1421, 1324 cm$^{-1}$;

$^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.81 (s, 1H), 7.76 (d, $J = 8.6$ Hz, 2H), 7.53 (d, $J = 8.6$ Hz, 2H), 7.48 (d, $J = 1.5$ Hz, 1H), 7.07 (dd, $J = 10.4$, 1.6 Hz, 1H), 6.70 (t, $J = 6.2$ Hz, 1H), 4.67 (s, 2H), 3.97 (d, $J = 2.5$ Hz, 2H), 3.25 (q, $J = 6.8$ Hz, 2H), 3.10 (s, 3H), 3.08 – 3.02 (m, 3H) ppm;
13C NMR (101 MHz, Acetone-d6) \( \delta \) 149.74 (d, \( J = 248.6 \) Hz), 140.67, 138.60, 133.73 (d, \( J = 6.3 \) Hz), 133.22, 129.91, 129.28, 124.03 (d, \( J = 13.0 \) Hz), 118.14 (d, \( J = 3.5 \) Hz), 113.01 (d, \( J = 2.1 \) Hz), 111.27 (d, \( J = 8.1 \) Hz), 110.96 (d, \( J = 20.0 \) Hz), 77.93, 76.75, 44.36, 42.39, 38.21, 36.75, 25.22 ppm;

HRMS (m/z): [M+Li]+ calcd. for C21H20BrClFN3O4S2Li, 580.9902; found, 580.9897.

(8d) was obtained as a yellow oil (50%, 0.035 g, 0.049 mmol).

IR (thin film): 3290, 3093, 2929, 2858, 1551, 1480, 1361, 1331, 1283, 1264 cm\(^{-1}\);

1H NMR (400 MHz, Chloroform-d) \( \delta \) 8.78 (s, 1H), 8.00 (d, \( J = 8.5 \) Hz, 1H), 7.73 – 7.64 (m, 4H), 7.44 – 7.37 (m, 2H), 7.29 – 7.26 (m, 1H), 7.06 (dd, \( J = 10.1, 1.6 \) Hz, 1H), 4.70 (s, 2H), 4.06 (d, \( J = 2.5 \) Hz, 2H), 3.21 (q, \( J = 6.5 \) Hz, 2H), 2.95 (t, \( J = 6.6 \) Hz, 3H), 2.32 (d, \( J = 2.4 \) Hz, 1H) ppm;

13C NMR (101 MHz, Chloroform-d) \( \delta \) 148.87 (d, \( J = 250.2 \) Hz), 148.52, 140.77, 139.41, 138.21, 132.30 (d, \( J = 13.7 \) Hz), 131.58, 130.61 (d, \( J = 5.4 \) Hz), 129.54, 128.42, 124.93, 123.50 (d, \( J = 13.2 \) Hz), 117.21 (d, \( J = 3.5 \) Hz), 112.39 (d, 2.02 Hz), 112.06, 112.39 (d, \( J = 1.9 \) Hz), 111.97 (d, \( J = 19.9 \) Hz), 76.49, 75.34, 43.20, 41.86, 36.91, 24.98 ppm;

HRMS (m/z): [M+Li]+ calcd. for C26H20BrCl2FN3O4SLi, 721.9520; found, 721.9516.
(8e) was obtained as a white solid (65%, 0.063 g, 0.096 mmol).

**Melting point:** 180.2 – 181.5 °C;

**IR (thin film):** 3279, 3223, 2959, 2918, 2858, 1573, 1486, 1451, 1354, 1328 cm⁻¹;

**¹H NMR (400 MHz, Acetone-d₆)** δ 10.70 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 1.5 Hz, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.05 (dd, J = 10.5, 1.6 Hz, 1H), 6.66 (t, J = 6.2 Hz, 1H), 4.58 (s, 2H), 4.00 (d, J = 2.6 Hz, 2H), 3.19 (q, J = 6.9 Hz, 2H), 2.95 (t, J = 7.1 Hz, 2H), 2.53 (s, 1H) ppm;

**¹³C NMR (101 MHz, Acetone-d₆)** δ 149.76 (d, J = 248.7 Hz), 144.86, 140.76, 138.62, 136.55, 133.75 (d, J = 6.2 Hz), 133.15, 130.49, 129.94, 129.31, 128.80, 124.03 (d, J = 13.1 Hz), 118.12 (d, J = 3.5 Hz), 112.99 (d, J = 1.9 Hz), 111.27 (d, J = 8.0 Hz), 110.95 (d, J = 20.2 Hz), 76.99, 76.16, 44.32, 42.66, 37.08, 25.31, 21.48 ppm;

**HRMS (m/z):** [M+Li]⁺ calcd. for C₂₇H₂₄BrClFN₃O₄S₂Li, 657.0215; found, 657.0217.

(8f) was obtained as a colorless oil (40%, 0.020 g, 0.032 mmol).
IR (thin film): 3272, 2929, 1745, 1693, 1577, 1458, 1421, 1372, 1324, 1249 cm⁻¹;

¹H NMR (400 MHz, Chloroform-d) δ 8.93 (s, 1H), 7.60 – 7.52 (m, 2H), 7.41 – 7.26 (m, 7H), 7.19 (s, 1H), 7.02 (dd, 7.19 J = 10.1, 1.6 Hz, 1H), 5.29 (s, 1H), 5.23 (s, 2H), 4.61 (s, 2H), 4.19 (s, 2H), 3.34 – 3.19 (m, 2H), 2.93 (s, 2H), 2.41 (s, 1H) ppm;

¹³C NMR (101 MHz, Chloroform-d) δ 156.56, 148.79 (d, 7.19 J = 249.3 Hz), 139.00, 138.24, 135.78, 133.94 (d, 7.19 J = 3.2 Hz), 131.99, 129.22, 128.82, 128.33, 128.15, 127.12, 123.01 (d, 7.19 J = 12.9 Hz), 122.88, 117.12 (d, 7.19 J = 3.2 Hz), 111.34 (d, 7.19 J = 19.8 Hz), 110.15, 78.99, 73.78, 68.60, 42.98, 42.40, 37.66, 24.58 ppm;

HRMS (m/z): [M+Li]⁺ calcd. for C₂₈H₂₄BrClF₅O₄SLi, 637.0494; found, 637.0488.

(8g) was obtained as a white solid (81%, 0.067 g, 0.101 mmol).

Melting point: 126.7 – 127.6 °C;

IR (thin film): 3327, 3305, 3104, 2962, 2918, 2854, 1536, 1447, 1350, 1320, 1257 cm⁻¹;

¹H NMR (400 MHz, Acetone-d₆) δ 10.32 (s, 1H), 8.45 (d, 7.19 J = 8.8 Hz, 2H), 8.22 (d, 7.19 J = 8.8 Hz, 2H), 7.76 (d, 7.19 J = 8.5 Hz, 2H), 7.60 (d, 7.19 J = 1.9 Hz, 1H), 7.52 (d, 7.19 J = 8.5 Hz, 2H), 7.34 (d, 7.19 J = 8.6 Hz, 1H), 7.21 (dd, 7.19 J = 8.6, 1.9 Hz, 1H), 6.67 (t, 7.19 J = 6.2 Hz, 1H), 4.68 (s, 2H), 4.05 (d, 7.19 J = 2.5 Hz, 2H), 3.18 (q, 7.19 J = 6.9 Hz, 2H), 2.95 (t, 7.19 J = 7.1 Hz, 2H), 2.62 (t, 7.19 J = 2.5 Hz, 1H) ppm;
$^{13}$C NMR (101 MHz, Acetone-$d_6$) $\delta$ 151.36, 145.01, 140.77, 138.65, 136.13, 131.07, 130.56, 130.31, 130.00, 129.33, 125.52, 125.17, 121.78, 114.05, 112.79, 112.39, 76.85, 76.34, 44.52, 42.90, 37.03, 25.31 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{26}$H$_{22}$BrClN$_4$O$_6$S$_2$Li, 670.0004; found, 669.9983.

(8h) was obtained as a yellow solid (64%, 0.115 g, 0.197 mmol).

Melting point: 69.6 – 71.1 °C;

IR (thin film): 3409, 3286, 3108, 3067, 2933, 1704, 1611, 1532, 1473, 1454, 1350, 1313 cm$^{-1}$;

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 8.58 (s, 1H), 8.36 (d, $J$ = 8.8 Hz, 2H), 8.07 (d, $J$ = 8.5 Hz, 2H), 7.53 (s, 1H), 7.37 – 7.26 (m, 5H), 7.17 (dd, $J$ = 8.6, 2.0 Hz, 1H), 5.05 (s, 2H), 4.84 (t, $J$ = 6.4 Hz, 1H), 4.49 (s, 2H), 4.05 (d, $J$ = 2.5 Hz, 2H), 3.41 (q, $J$ = 6.8 Hz, 2H), 2.91 (t, $J$ = 7.0 Hz, 2H), 2.21 – 2.09 (m, 1H) ppm;

$^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 156.37, 150.46, 144.45, 136.51, 134.57, 129.07, 129.00, 128.78, 128.68, 128.29, 128.05, 125.73, 124.51, 123.56, 118.50, 112.41, 75.90, 75.38, 66.79, 41.75, 41.41, 36.38, 29.84, 24.62 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{28}$H$_{25}$ClN$_4$O$_6$SLi, 586.1335; found, 586.1339.
(8i) was obtained as a yellow solid (60%, 0.111 g, 0.180 mmol).

**Melting point:** 63.8 – 65.1°C;

**IR (thin film):** 3406, 3286, 3108, 2933, 1704, 1611, 1532, 1477, 1458, 1350, 1316 cm⁻¹;

**¹H NMR** (400 MHz, Chloroform-­d) δ 8.61 (s, 1H), 8.33 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.45 (s, 1H), 7.38 – 7.28 (m, 6H), 7.22 (d, J = 1.7 Hz, 1H), 5.05 (s, 3H), 4.81 (s, 1H), 4.50 (s, 2H), 4.10 (d, J = 2.5 Hz, 2H), 3.40 (q, J = 6.8 Hz, 3H), 2.91 (t, J = 7.0 Hz, 2H), 2.21 (s, 1H) ppm;

**¹³C NMR** (101 MHz, Chloroform-­d) δ 156.34, 150.43, 144.36, 132.06, 129.92, 128.97, 128.71, 128.36, 128.10, 125.83, 124.46, 122.77, 117.38, 117.25, 113.97, 76.15, 75.56, 66.85, 41.69, 41.59, 36.86, 24.77 ppm;

**HRMS (m/z):** [M+Li]⁺ calcd. for C₂₈H₂₄Cl₂N₄O₆SLi, 620.0945; found, 620.0950.

(8j) was obtained as a yellow oil (92 %, 0.089 g, 0.158 mmol).

**IR (thin film):** 3413, 3286, 3108, 3067, 2929, 1704, 1532, 1491, 1458, 1350, 1313 cm⁻¹;
\[ ^1H \text{NMR} \ (400 \text{ MHz, Acetone-}d_6 \) \delta 10.19 \ (s, \ 1H), \ 8.44 \ (d, \ J = 8.8 \text{ Hz, } 2H), \ 8.22 \ (d, \ J = 8.8 \text{ Hz, } 2H), \ 7.39 \ (dd, \ J = 8.8, 4.5 \text{ Hz, } 1H), \ 7.36 - 7.25 \ (m, \ 6H), \ 6.92 \ (td, \ J = 9.2, 2.5 \text{ Hz, } 1H), \ 6.45 \ (t, \ J = 6.3 \text{ Hz, } 1H), \ 5.04 \ (s, \ 2H), \ 4.69 \ (s, \ 2H), \ 4.10 \ (d, \ J = 2.5 \text{ Hz, } 2H), \ 3.37 \ (q, \ J = 6.8 \text{ Hz, } 2H), \ 2.96 \ (t, \ J = 7.3 \text{ Hz, } 2H), \ 2.66 \ (t, \ J = 2.5 \text{ Hz, } 1H) \text{ ppm;} \]

\[ ^{13}C \text{NMR} \ (101 \text{ MHz, Acetone-}d_6 \) \delta 157.19, \ 151.31, \ 145.15, \ 138.67, \ 138.44, \ 134.09, \ 131.02, \ 130.25, \ 129.17, \ 128.52, \ 128.50, \ 125.13, \ 113.74 \ (d, \ J = 5.1 \text{ Hz}), \ 113.06 \ (d, \ J = 9.6 \text{ Hz}), \ 111.04, \ 110.78, \ 104.28 \ (d, \ J = 23.7 \text{ Hz}), \ 76.83, \ 76.50, \ 66.36, \ 42.81, \ 42.42, \ 37.02, \ 25.28 \text{ ppm;} \]

HRMS (m/z): \([\text{M+Li}]^+\) calcd. for C_{28}H_{25}FN_4O_6SLi, 570.1630; found, 570.1624.

Gold-Catalyzed Cyclization Procedure for 9a-j and Analytical Data:

*General Procedure G (Gold-Catalyzed Tandem Cyclization):*

8a (40.0 mg, 0.058 mmol) was dissolved in anhydrous 1,4-dioxane (1.0 mL) in a sealed tube. 5 mol % of the catalyst XPhosAuNTf_2 (2.8 mg, 2.9 \mu\text{mol}) was added as a solid and the reaction was heated at 90 °C for 12 hours under argon atmosphere. The reaction mixture was then concentrated in vacuo with diatomaceous earth. The free flowing powder was used directly for column chromatography on silica gel eluting with 80:20 hexanes:ethyl acetate to afford tetracyclic indoline 9a as a clear oil (97%, 39.0 mg, 0.057 mmol).
**IR (thin film):** 3327, 3108, 2936, 2866, 1730, 1626, 1599, 1532, 1480, 1428, 1402, 1305 cm\(^{-1}\);

**\(^1\)H NMR (400 MHz, Chloroform-\(d\))** \(\delta\) 8.39 (d, \(J = 8.7\) Hz, 2H), 8.01 (d, \(J = 8.7\) Hz, 2H), 7.81 – 7.71 (m, 2H), 7.47 (d, \(J = 8.5\) Hz, 2H), 6.97 (d, \(J = 1.6\) Hz, 0H), 6.84 (s, 1H), 5.21 (s, 1H), 5.10 (s, 1H), 4.71 (s, 1H), 4.24 (d, \(J = 14.0\) Hz, 1H), 3.97 (d, \(J = 13.1\) Hz, 1H), 3.60 (dd, \(J = 22.2, 13.5\) Hz, 2H), 3.37 (t, \(J = 8.1\) Hz, 1H), 2.99 – 2.89 (m, 1H), 2.40 – 2.28 (m, 1H), 2.22 (dd, \(J = 12.7, 5.8\) Hz, 1H) ppm;

**\(^{13}\)C NMR (75 MHz, Chloroform-\(d\))** \(\delta\) 150.43, 147.63 (d, \(J = 247.6\) Hz), 143.80, 140.24, 140.05, 136.72, 134.69 (d, \(J = 13.0\) Hz), 132.99 (d, \(J = 4.7\) Hz), 129.61, 129.15, 128.64, 124.56, 122.79 (d, \(J = 3.5\) Hz), 119.59 (d, \(J = 20.4\) Hz), 116.64, 110.87 (d, \(J = 7.1\) Hz), 90.09, 62.26 (d, \(J = 2.0\) Hz), 50.61, 48.43, 47.58, 33.50 ppm;

**HRMS (m/z):** [M+Li]\(^+\) calcd. for C\(_{26}\)H\(_{21}\)BrClFN\(_4\)O\(_6\)S\(_2\)Li, 687.9910; found, 687.9907.

(9b) was obtained as a yellow oil (72 %, 0.014 g, 0.024 mmol).

**IR (thin film):** 3361, 3096, 2929, 1693, 1629, 1529, 1480, 1402, 1343 cm\(^{-1}\);
\(^1\text{H NMR}\) (400 MHz, Chloroform-\(d\)) \(\delta\) 7.72 (d, \(J = 8.5\) Hz, 2H), 7.46 (d, \(J = 8.1\) Hz, 2H), 7.00 (d, \(J = 9.5\) Hz, 1H), 6.85 (s, 1H), 5.27 (s, 1H), 5.25 (s, 1H), 5.15 (s, 1H), 5.00 (s, 1H), 4.76 (d, \(J = 14.2\) Hz, 1H), 4.00 (d, \(J = 15.4\) Hz, 1H), 3.82 (d, \(J = 15.3\) Hz, 1H), 3.60 – 3.36 (m, 3H), 3.08 – 2.90 (m, 1H), 2.50 – 2.33 (m, 2H) ppm;

\(^{13}\text{C NMR}\) (101 MHz, Chloroform-\(d\)) \(\delta\) 157.53, 147.96 (d, \(J = 247.9\) Hz), 141.39, 139.58, 138.08, 137.16 (d, \(J = 13.2\) Hz), 134.25 (d, \(J = 4.8\) Hz), 129.66, 128.49, 122.13 (d, \(J = 3.2\) Hz), 119.51 (d, \(J = 8.5\) Hz), 115.97, 110.30 (d, \(J = 4.1\) Hz), 65.20, 52.30 (d, \(J = 1.9\) Hz), 50.74, 45.81, 39.33, 37.75, 29.84 ppm;

\textbf{HRMS (m/z):} \([\text{M+Li}]^+\) calcd. for C\(_{22}\)H\(_{17}\)BrCl\(_2\)F\(_4\)N\(_2\)O\(_3\)SLi, 598.9950; found, 598.9960.

\((9c)\) was obtained as a colorless oil (90\%, 0.036 g, 0.062 mmol).

\textbf{IR (thin film):} 3368, 3096, 3022, 2929, 2858, 1629, 1592, 1480, 1417, 1398, 1339 cm\(^{-1}\);

\(^1\text{H NMR}\) (400 MHz, Chloroform-\(d\)) \(\delta\) 7.78 (d, \(J = 8.6\) Hz, 2H), 7.45 (d, \(J = 8.6\) Hz, 2H), 7.04 (dd, \(J = 9.4, 1.7\) Hz, 1H), 6.92 (d, \(J = 1.7\) Hz, 1H), 5.28 (s, 1H), 5.22 (s, 1H), 5.08 (s, 1H), 4.32 (d, \(J = 14.1\), 1H), 3.95 – 3.84 (m, 3H), 3.60 (d, \(J = 14.1\) Hz, 1H), 3.46 (ddd, \(J = 9.0, 7.5, 1.5\) Hz, 1H), 2.95 (ddd, \(J = 11.1, 9.0, 5.9\) Hz, 1H), 2.87 (s, 3H), 2.43 (ddd, \(J = 12.6, 11.1, 7.5\) Hz, 1H), 2.21 (ddd, \(J = 12.6, 5.9, 1.5\) Hz, 1H) ppm;
$^{13}$C NMR (101 MHz, Chloroform-$d$) δ 148.23 (d, $J = 247.9$ Hz), 140.72, 139.87, 136.82, 134.94 (d, $J = 13.4$ Hz), 133.79 (d, $J = 4.9$ Hz), 129.52, 129.21, 122.99 (d, $J = 3.2$ Hz), 119.55 (d, $J = 20.4$ Hz), 116.27, 111.05 (d, $J = 7.0$ Hz), 90.15, 62.17 (d, $J = 1.8$ Hz), 50.88, 48.33, 47.70, 38.53, 33.28 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{21}$H$_{20}$BrClFN$_3$O$_4$S$_2$Li, 580.9902; found, 580.9908.

(9d) was obtained as a colorless oil (91%, 0.024 g, 0.033 mmol).

IR (thin film): 3376, 3096, 2925, 2854, 1629, 1551, 1480, 1343, 1283, 1242 cm$^{-1}$;

$^1$H NMR (500 MHz, Chloroform-$d$) δ 7.95 (d, $J = 8.4$ Hz, 1H), 7.79 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 8.2$ Hz, 2H), 6.98 (d, $J = 9.4$ Hz, 1H), 6.85 (s, 1H), 5.26 (s, 1H), 5.13 (s, 1H), 5.08 (s, 1H), 4.37 (d, $J = 14.2$ Hz, 1H), 4.01 (d, $J = 13.5$ Hz, 1H), 3.86 (d, $J = 13.4$ Hz, 1H), 3.58 (d, $J = 14.2$ Hz, 1H), 3.46 (t, $J = 8.2$ Hz, 1H), 2.98 – 2.91 (m, 1H), 2.50 – 2.39 (m, 1H), 2.26 – 2.16 (m, 1H) ppm;

$^{13}$C NMR (75 MHz, Chloroform-$d$) δ 140.49, 140.47 140.34, 139.89, 136.89, 135.17, 132.36, 132.03, 130.74, 129.52, 129.32, 124.57, 122.78 (d, $J = 3.5$ Hz), 122.75, 119.53 (d, $J = 20.4$ Hz), 116.45, 89.99, 77.36, 62.54 (d, $J = 2.0$ Hz), 50.69, 48.52, 47.67, 33.52, 29.86 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{26}$H$_{20}$BrCl$_3$FN$_4$O$_6$S$_2$Li, 721.9520; found, 721.9518.
(9e) was obtained as a colorless oil (96%, 0.038 g, 0.059 mmol).

**IR (thin film):** 3372, 3093, 3029, 2929, 2862, 1726, 1629, 1599, 1480, 1398, 1339 cm⁻¹;

**¹H NMR (400 MHz, Chloroform-d)** δ 7.82 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 6.98 (dd, J = 9.5, 1.7 Hz, 1H), 6.84 (d, J = 1.7 Hz, 1H), 5.15 (d, J = 1.4 Hz, 1H), 5.04 (s, 1H), 4.74 (s, 1H), 4.03 (d, J = 13.7 Hz, 1H), 3.91 (d, J = 13.1 Hz, 1H), 3.63 – 3.50 (m, 2H), 3.40 – 3.27 (m, 1H), 3.01 – 2.86 (m, 1H), 2.47 (s, 3H), 2.42 – 2.29 (m, 1H), 2.23 – 2.12 (m, 1H) ppm;

**¹³C NMR (101 MHz, Chloroform-d)** δ 144.34, 140.77, 139.84, 136.88, 135.07, 134.43, 133.39 (d, J = 5.0 Hz), 130.06, 129.47, 129.43, 127.49, 122.75 (d, J = 3.2 Hz), 119.48 (d, J = 20.4 Hz), 119.37, 115.96, 110.53, 90.44, 62.35 (d, J = 1.8 Hz), 50.73, 48.50, 47.60, 33.54, 21.74 ppm;

**HRMS (m/z):** [M+ Na]⁺ calcd. for C₂₇H₂₄BrClFN₃O₅S₂Na, 673.9962; found, 673.9975.

(9f) was obtained as a clear oil (71%, 0.015 g, 0.024 mmol).
**IR (thin film):** 3383, 3093, 3070, 2929, 2880, 1704, 1592, 1480, 1443, 1417, 1376 cm\(^{-1}\);

**\(^1\)H NMR (400 MHz, Chloroform-\(d\))\:** \(\delta 7.81\) (d, \(J = 8.2\) Hz, 2H), 7.46–7.28 (m, 7H), 6.96 (d, \(J = 9.5\) Hz, 1H), 6.84 (s, 1H), 5.17 (d, \(J = 19.0\) Hz, 1H), 5.09 (s, 1H), 5.04 (d, \(J = 19.0\) Hz, 1H), 4.80 (s, 1H), 4.69 (d, \(J = 14.0\) Hz, 1H), 4.55 (d, \(J = 14.3\) Hz, 1H), 4.02–3.97 (m, 1H), 3.89–3.80 (m, 1H), 3.62–3.36 (m, 2H), 2.93–2.81 (m, 1H), 2.47–2.31 (m, 1H), 2.25–2.13 (m, 1H) ppm;

**\(^{13}\)C NMR (101 MHz, Chloroform-\(d\))\:** \(\delta 156.07, 147.56\) (d, \(J = 246.5\) Hz), 142.44, 139.61 (d, \(J = 4.0\) Hz), 137.33, 136.53 (d, \(J = 2.4\) Hz), 135.85, 133.75 (d, \(J = 3.9\) Hz), 129.43, 128.79, 127.81, 122.85 (d, \(J = 2.9\) Hz), 119.34 (d, \(J = 20.4\) Hz), 115.20, 110.11 (d, \(J = 8.1\) Hz), 90.56, 67.55, 62.66 (d, \(J = 2.1\) Hz), 49.12, 48.79, 47.97, 47.47, 34.04, 29.84 ppm.

**HRMS (m/z):** [M+Li]\(^+\) calcd. for C\(_{28}\)H\(_{24}\)BrClFN\(_3\)O\(_4\)SLi, 637.0494; found, 637.0507.

\((9g)\) was obtained as a yellow oil (92%, 0.027 g, 0.040 mmol).

**IR (thin film):** 3379, 3104, 2925, 2858, 1722, 1611, 1592, 1532, 1480, 1402, 1350 cm\(^{-1}\);

**\(^1\)H NMR (400 MHz, Chloroform-\(d\))\:** \(\delta 8.38\) (d, \(J = 8.8\) Hz, 1H), 8.01 (d, \(J = 8.8\) Hz, 1H), 7.81 (d, \(J = 8.7\) Hz, 1H), 7.47 (d, \(J = 8.6\) Hz, 1H), 7.14 (dd, \(J = 8.3, 2.0\) Hz, 1H), 7.03 (d, \(J = 2.0\) Hz, 1H), 6.24 (d, \(J = 8.3\) Hz, 1H), 5.16 (s, 1H), 5.09 (s, 1H), 4.79 (s, 1H), 4.11 (d, \(J = 13.5\) Hz, 1H), 3.95 (d, \(J = 13.1\) Hz, 1H), 3.68–3.59 (m, 2H), 3.37–3.23 (m, 1H), 3.10–2.94 (m, 1H), 2.42–2.28 (m, 1H), 2.26–2.16 (m, 1H) ppm;
$^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 150.32, 147.32, 143.81, 140.54, 139.98, 137.09, 132.44, 130.28, 129.54, 129.32, 128.71, 127.10, 124.58, 116.23, 111.58, 110.96, 89.95, 61.83, 50.72, 48.65, 47.55, 33.71 ppm;

HRMS (m/z): [M+Na]$^+$ calcd. for C$_{26}$H$_{22}$BrClN$_4$O$_6$S$_2$Na, 686.9750; found, 686.9743.

(9h) was obtained as a yellow solid (96%, 0.036 g, 0.062 mmol).

Melting point: 77.6 – 79.1 °C;

IR (thin film): 3376, 3104, 3033, 2929, 2877, 1693, 1611, 1532, 1480, 1458, 1406, 1350 cm$^{-1}$;

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 8.32 (d, $J$ = 8.7 Hz, 2H), 7.97 (d, $J$ = 8.7 Hz, 2H), 7.41 – 7.30 (m, 5H), 7.10 – 6.88 (m, 2H), 6.30 (d, $J$ = 8.2 Hz, 1H), 5.18 – 5.15 (m, 1H), 5.13 – 5.00 (m, 3H), 4.20 (d, $J$ = 13.5 Hz, 1H), 3.90 – 3.77 (m, 2H), 3.69 – 3.59 (m, 2H), 3.20 – 3.05 (m, 1H), 2.39 – 2.31 (m, 1H), 2.25 – 2.14 (m, 1H) ppm;

$^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 154.48, 150.10, 147.27, 144.35, 140.84, 136.03, 130.32, 129.29, 128.74, 128.67, 128.40, 127.92, 124.76, 124.35, 116.06, 110.89, 87.68, 67.24, 60.48, 48.85, 48.79, 46.50, 33.25 ppm;

HRMS (m/z): [M+Li]$^+$ calcd. for C$_{28}$H$_{25}$ClN$_4$O$_6$SLi, 587.1344; found, 587.1376.
(9i) was obtained as a yellow solid (95%, 0.060 g, 0.097 mmol).

**Melting point:** 87.7 – 89.5 °C

**IR (thin film):** 3379, 3104, 2955, 1693, 1691, 1588, 1532, 1465, 1406, 1350, 1313, 1242 cm\(^{-1}\);

\(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 8.34 (d, \(J = 8.8 \text{ Hz}, 2\)H), 7.98 (d, \(J = 8.8 \text{ Hz}, 2\)H), 4.09 – 4.01 (m, 5H), 7.05 (d, \(J = 2.0 \text{ Hz}, 1\)H), 6.89 (d, \(J = 2.0 \text{ Hz}, 1\)H), 5.24 – 5.15 (m, 3H), 5.09 (s, 1H), 4.21 (d, \(J = 13.0 \text{ Hz}, 1\)H), 4.04 (d, \(J = 14.1 \text{ Hz}, 1\)H), 3.74 (d, \(J = 14.1 \text{ Hz}, 1\)H), 3.68 – 3.61 (m, 1H), 3.59 (d, \(J = 13.0 \text{ Hz}, 1\)H), 3.19 – 3.06 (m, 1H), 2.43 – 2.11 (m, 2H) ppm;

\(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 154.22, 150.14, 144.32, 143.99, 140.54, 135.75, 131.22, 129.46, 129.11, 128.80, 128.72, 128.30, 128.54, 127.99, 124.35, 124.14, 122.96, 115.59, 87.12, 67.26, 61.15, 48.28, 46.31, 33.17 ppm;

**HRMS (m/z):** [M+Li]\(^+\) calcd. for C\(_{28}\)H\(_{24}\)Cl\(_2\)N\(_4\)O\(_{6}\)SLi, 621.0954; found, 621.0959.

(9j) was obtained as a yellow oil (91%, 0.019 g, 0.032 mmol).

**IR (thin film):** 3376, 3104, 3037, 2925, 2854, 1693, 1611, 1532, 1491, 1451, 1406, 1350 cm\(^{-1}\);
\textbf{1H NMR} (400 MHz, Chloroform-\textit{d}) $\delta$ 8.30 (d, $J = 8.8$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.38 (s, 1H), 7.38 – 7.28 (m, 6H), 6.80 – 6.70 (m, 1H), 6.32 – 6.24 (m, 1H), 5.14 (s, 1H), 5.10 – 4.97 (m, 3H), 4.19 (d, $J = 13.2$ Hz, 1H), 3.81 (s, 2H), 3.70 – 3.61 (m, 1H), 3.18 – 3.01 (m, 1H), 2.43 – 2.09 (m, 2H) ppm;

\textbf{13C NMR} (101 MHz, Chloroform-\textit{d}) $\delta$ 157.27 (d, $J = 237.6$ Hz), 154.44, 150.10, 144.38, 140.93, 136.07, 129.92 (d, $J = 7.8$ Hz), 128.74, 128.48, 128.39, 127.92, 124.32, 124.11, 115.94, 111.89 (d, $J = 24.1$ Hz), 111.77, 110.47 (d, $J = 8.0$ Hz), 88.01, 60.60, 49.03, 48.83, 46.52, 33.22, 29.85 ppm;

\textbf{HRMS} (m/z): [M+Li]$^+$ calcd. for C$_{28}$H$_{25}$FN$_4$O$_6$SLi, 571.1639; found, 571.1636.

**Preparation of Aza-Tricyclic Indolines (11a-c, 12-14) and Analytical Data:**

\textit{General Procedure H (Reductive Ring-Opening Reaction):}

A solution of TFA (1.0 mmol, 0.114 g, 0.077 mL) was prepared in THF (1.0 mL); 2 equivalents of TFA from this solution (0.117 mmol, 0.117 ml, 1.0 M in THF) were added to a solution of 9a (0.040 g, 0.058 mmol, 0.10 M) in a 10:1 mixture of THF:MeOH (0.580 mL) at 0 °C. The reaction was warmed to ambient temperature and stirred for 1 hour. The reaction was concentrated from methanol three times then dissolved in ethyl acetate and the organic layer was washed with a saturated aqueous solution of NaHCO$_3$. The combined organic layers were dried
over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel with 60:40 hexanes:ethyl acetate to afford 11a as a yellow oil (92%, 0.037 g, 0.054 mmol).

(11a).

**IR (thin film):** 3353, 3104, 2925, 2866, 1532, 1480, 1402, 1350, 1316 cm$^{-1}$;

**$^1$H NMR** (500 MHz, Chloroform-$d$) $\delta$ 8.33 (d, $J = 8.7$ Hz, 2H), 7.87 (d, $J = 8.7$ Hz, 2H), 7.70 (d, $J = 8.6$ Hz, 2H), 7.48 (d, $J = 8.6$ Hz, 2H), 6.95 (dd, $J = 9.4$, 1.6 Hz, 1H), 6.66 (d, $J = 1.6$ Hz, 1H), 5.25 (s, 1H), 5.16 (s, 1H), 4.94 (t, $J = 6.0$ Hz, 1H), 3.94 (s, 1H), 3.92 (t, $J = 3.5$ Hz, 1H), 3.83 (d, $J = 13.1$ Hz, 1H), 3.66 (d, $J = 13.1$ Hz, 1H), 3.37 (dd, $J = 13.8$, 3.8 Hz, 1H), 3.27 (dd, $J = 13.7$, 3.5 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.81 – 2.69 (m, 1H), 2.13 – 1.92 (m, 2H) ppm;

**$^{13}$C NMR** (75 MHz, Chloroform-$d$) $\delta$ 150.22, 148.00 (d, $J = 246.8$ Hz), 143.54, 140.72, 139.52, 137.95, 136.49 (d, $J = 13.0$ Hz), 134.91 (d, $J = 4.8$ Hz), 129.63, 128.41 (d, $J = 9.5$ Hz), 124.51, 122.28 (d, $J = 3.2$ Hz), 118.99 (d, $J = 20.5$ Hz), 115.66, 109.99 (d, $J = 7.1$ Hz), 63.90, 52.15, 52.12, 50.18, 47.71, 39.28, 37.31 ppm;

**HRMS (m/z):** [M+Li]$^+$ calcd. For C$_{26}$H$_{23}$BrClFN$_4$O$_6$S$_2$Li, 691.0076; found, 691.0068.

General Procedure H was used to Prepare 11b and 11c.
(11b) was obtained as a colorless oil (75% yield, 13.6 mg, 0.023 mmol).

**IR (thin film):** 3350, 3238, 3108, 2925, 2854, 1477, 1410, 1316, 1290 cm\(^{-1}\);

\(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 7.77 – 7.65 (m, 2H), 7.52 – 7.43 (m, 2H), 7.04 (dd, \(J = 9.5, 1.7\) Hz, 1H), 6.78 (d, \(J = 1.7\) Hz, 1H), 5.25 (d, \(J = 1.5\) Hz, 1H), 5.10 (d, \(J = 1.1\) Hz, 1H), 5.00 (t, \(J = 6.3\) Hz, 1H), 4.17 – 4.06 (m, 1H), 3.93 – 3.85 (m, 2H), 3.78 (d, \(J = 13.7\) Hz, 1H), 3.47 (dd, \(J = 14.1, 3.8\) Hz, 1H), 3.35 (dd, \(J = 14.1, 3.5\) Hz, 1H), 3.00 – 2.80 (m, 2H), 2.77 (s, 3H), 2.20 – 1.93 (m, 2H) ppm;

\(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 148.51 (d, \(J = 247.4\) Hz), 141.62, 139.54, 138.07, 136.67 (d, \(J = 13.0\) Hz), 135.41 (d, \(J = 4.5\) Hz), 129.67, 128.56, 122.60 (d, \(J = 3.0\) Hz), 119.12 (d, \(J = 20.5\) Hz), 115.44, 110.36 (d, \(J = 6.7\) Hz), 64.18, 52.22, 49.96, 47.25, 39.55, 37.69, 37.04 ppm;

**HRMS (m/z):** [M+Li]\(^+\) calcd. for C\(_{21}\)H\(_{22}\)BrClF\(_3\)N\(_3\)O\(_4\)S\(_2\)Li, 587.0068; found, 587.0032.

(11c) was obtained as a yellow oil (89%, 0.013 g, 0.022 mmol).

**IR (thin film):** 3342, 3275, 2925, 2854, 1681, 1625, 1592, 1480, 1331, 1298 cm\(^{-1}\);
\[ ^1H \text{NMR} \] (400 MHz, Chloroform-\(d \)) \( \delta \) 7.67 (d, \( J = 8.5 \) Hz, 2H), 7.45 (d, \( J = 8.5 \) Hz, 2H), 7.00 (dd, \( J = 9.5, 1.7 \) Hz, 1H), 6.70 (d, \( J = 1.7 \) Hz, 1H), 5.32 (s, 1H), 5.24 (s, 1H), 5.00 (s, 1H), 4.27 (d, \( J = 14.3 \) Hz, 1H), 4.17 – 3.86 (m, 3H), 3.10 (d, \( J = 14.3 \) Hz, 1H), 2.93 – 2.67 (m, 2H), 2.23 – 1.98 (m, 2H) ppm;

\[ ^{13}C \text{NMR} \] (101 MHz, Chloroform-\(d \)) \( \delta \) 157.36, 147.79 (d, \( J = 247.9 \) Hz), 141.22, 140.66, 139.37 (d, \( J = 7.6 \) Hz), 137.90 (d, \( J = 2.3 \) Hz), 136.99 (d, \( J = 13.2 \) Hz), 134.11, 129.50, 128.33, 121.97 (d, \( J = 3.2 \) Hz), 119.29 (d, \( J = 20.2 \) Hz), 115.81, 110.08 (d, \( J = 7.1 \) Hz), 65.04, 52.48 (d, \( J = 1.7 \) Hz), 50.57, 46.87, 39.16, 29.68 ppm;

HRMS (m/z): [M+Li]\(^+\) calcd. for C\(_{22}\)H\(_{19}\)BrClF\(_4\)N\(_3\)O\(_3\)SLi, 601.0106; found, 601.0103.

Thiophenol (0.045 ml, 0.50 mmol) and K\(_2\)CO\(_3\) (0.057 g, 0.50 mmol) were added to a solution of 11a (0.057 g, 0.083 mmol, 0.080 M) in anhydrous ACN. The reaction was refluxed under argon for 3 hours. The reaction was cooled to room temperature and the solvent was removed in vacuo to give a residue, which was dissolved in methanol then concentrated onto diatomaceous earth to give a free-flowing solid which was loaded onto an equilibrated* silica gel chromatography column eluting with 90:8:2 DCM:MeOH:TEA to give 12 as a clear oil (84% yield, 0.035 g, 0.070 mmol).

*Equilibration conditions Flush with 100% methanol followed by equilibration with DCM.
IR (thin film): 3279, 3089, 2936, 2862, 1625, 1592, 1476, 1327 cm\(^{-1}\);

\(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 7.77 (d, \(J = 8.5\) Hz, 2H), 7.50 (d, \(J = 8.5\) Hz, 2H), 7.06 (dd, \(J = 9.4, 1.7\) Hz, 1H), 6.89 (d, 7.17 Hz, 1H), 5.17 (s, 1H), 5.04 (s, 1H), 4.79 (s, 1H), 4.03 (s, 1H), 3.78 (s, 1H), 3.43 (d, \(J = 14.3\) Hz, 1H), 3.36 (d, \(J = 14.3\) Hz, 1H), 3.27 – 3.16 (m, 2H), 3.12 – 3.01 (m, 2H), 3.02 – 2.88 (m, 2H), 2.21 – 2.12 (m, 1H), 2.09 – 1.97 (m, 1H) ppm;

\(^13\)C NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 149.28 (d, \(J = 246.6\) Hz), 143.61, 139.40, 137.91, 137.07 (d, \(J = 4.8\) Hz), 136.31 (d, \(J = 13.4\) Hz), 129.62, 128.68, 122.86, 118.72 (d, \(J = 21.5\) Hz), 110.11 (d, \(J = 7.2\) Hz), 63.34, 51.99, 48.80, 45.40, 39.99, 34.47, 29.85 ppm;

HRMS (m/z): [M+H]\(^+\) calcd. for C\(_{20}\)H\(_{21}\)BrClF\(_3\)N\(_3\)O\(_2\)S, 500.0210; found, 500.0212.

A solution of \(\textbf{12}\) (0.011 g, 0.022 mmol, 0.070 M) was prepared in DCM (0.314 mL) and cooled to 0\(^\circ\)C. To this solution, \(N,N\)-di-Boc-1H-pyrazole-1-carboxamidine (0.024 mmol, 7.5 mg) was added and the reaction was warmed to room temperature with stirring under argon for 12 hours. The crude reaction was diluted to 1 mL with DCM then washed with 2.0 N NaOH then brine. The organic layer was dried with Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to give a clear residue which
was purified by column chromatography eluting with 70:30 hexanes: ethyl acetate to give diBoc-Guanidine ATI which was used directly in the next step. DiBoc-Guanidine ATI was dissolved in 1 mL 1:1 DCM:TFA at 0°C under argon and the reaction stirred for 4 hours. The reaction was then concentrated in vacuo to give 13 as a white solid (90% yield for 2 steps calculated based on 12, 0.013 g, 0.020 mmol).

(13)

**Melting point:** 98.9 – 100.5 °C;

**IR (thin film):** 3357, 3171, 2925, 1659, 1614, 1480, 1432, 1327, 1204 cm⁻¹;

**¹H NMR** (400 MHz, Methanol-d⁴) δ 7.70 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 9.8 Hz, 1H), 6.76 (s, 1H), 5.33 (s, 1H), 5.28 (s, 1H), 4.02 (s, 1H), 3.91 (d, J = 13.4 Hz, 1H), 3.77 (d, J = 13.2 Hz, 1H), 3.63 (d, J = 14.4 Hz, 1H), 3.25 (d, J = 14.4 Hz, 1H), 3.05 – 2.90 (m, 1H), 2.56 – 2.42 (m, 1H), 2.12 – 2.02 (m, 2H) ppm;

**¹³C NMR** (101 MHz, Methanol-d⁴) δ 158.15, 149.06 (d, J = 244.5 Hz), 142.90, 140.11, 139.85, 138.98 (d, J = 12.8 Hz), 136.70 (d, J = 5.0 Hz), 130.39, 129.66, 123.55 (d, J = 3.03 Hz), 119.66 (d, J = 20.0 Hz), 115.70, 109.53 (d, J = 6.9 Hz) 64.87, 53.41, 51.40, 47.48, 39.64, 38.06 ppm;

**HRMS (m/z):** [M+H]⁺ calcd. for C₂₁H₂₃BrClFN₅O₂S, 542.0428; found, 542.0427.
A solution of 12 (0.024 g, 0.048 mmol, 0.070 M) was prepared in DCM (0.686 mL) and cooled to 0°C under argon. A solution of tert-butylbromoacetate (1.0 mmol, 0.195 g, 0.148 mL) in DCM (1 mL) was prepared. One equivalent (0.048 ml, 0.048 mmol, 1.0 M in DCM) of this solution and TEA (0.020 mL, 0.144 mmol) were then added to the solution of 12 dropwise at 0°C. The reaction was then stirred for 3 hours at room temperature. The organic layer was diluted with DCM and washed with 2.0 N NaOH followed by brine. The organic layer was dried over Na₂SO₄ and then concentrated in vacuo to give a residue which was purified by column chromatography eluting with 50:48:2 Hex:EtOAc:TEA to give S17 which was used in the next step directly.

S17 was dissolved in 1.0 mL DCM:TFA 1:1 and cooled to 0°C followed by stirring under argon for 2 hours. The reaction was then concentrated in vacuo to give a yellow residue 13 as a yellow oil (38% yield for 2 steps, 0.012 g, 0.018 mmol).

(14)

IR (thin film): 3320, 2925, 2858, 1733, 1670, 1476, 1428, 1331 cm⁻¹;

¹H NMR (500 MHz, Methanol-d₄) δ 7.79 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.17 (dd, J = 9.7, 1.5 Hz, 1H), 7.12 (s, 1H), 5.39 (s, 1H), 5.13 (s, 1H), 4.30 – 4.06 (m, 3H), 4.01 (d, J = 13.7 Hz, 1H), 3.91 (d, J = 13.1 Hz, 1H), 3.86 – 3.73 (m, 1H), 3.59 (d, J = 13.3 Hz, 1H), 3.07 – 2.95 (m, 1H), 2.83 – 2.69 (m, 1H), 2.34 – 2.20 (m, 1H), 2.13 – 2.05 (m, 1H);

¹³C NMR (101 MHz, Methanol-d₄) δ 167.43, 150.63 (d, J = 246.4 Hz), 139.99 (d, J = 3.2 Hz), 137.54 (d, J = 5.4 Hz), 137.40 (d, J = 13.9 Hz), 130.52, 129.78, 124.50 (d, J = 2.5 Hz), 121.85,
119.83 (d, $J = 20.8$ Hz), 111.01 (d, $J = 7.2$ Hz), 62.87, 56.52, 52.28, 52.16, 47.85, 40.58, 34.63, 30.75, 28.17 ppm;

**HRMS (m/z):** [M+H]$^+$ calcd. for $\text{C}_{22}\text{H}_{23}\text{BrClFNN}_3\text{O}_4\text{S}$, 558.0266; found, 558.0264.

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A.1 Biological and Physiochemical Assay Protocols and Results For Chapter 3

**Microdilution tests for minimal inhibitory concentration (MIC) determination:**

The minimal inhibitory concentrations (MICs) of antimicrobial compounds were determined by the broth microdilution method detailed in the Clinical and Laboratory Standards Institute (CLSI) handbook. All antimicrobial compounds were purchased from Sigma-Aldrich. The growth media used for all MIC experiments was Mueller Hinton Broth (MHB) purchased from HIMEDIA through VWR (cat: 95039-356). The inoculum was prepared by diluting a bacterial day culture (OD$_{600}$ 0.15-0.4) to OD$_{600}$ 0.002. This dilution was further diluted two fold when added to 96 well microplates (USA Scientific CytoOne 96-well TC plate, cat: CC7682-7596) for a final inoculum concentration of OD$_{600}$ 0.001. All plates were incubated at 37°C with shaking for 18 hours before results were interpreted.

**Minimal re-sensitizing concentration (MRC) determination:**

Antibiotic MIC values where *S. aureus* is considered susceptible were determined from the CLSI handbook. MHB was supplemented with the antimicrobial at a concentration two fold
greater than the CLSI susceptible MIC value. Two-fold serial dilutions of 13 were prepared in antibiotic supplemented media in 96-well microplates. These were inoculated with MRSA diluted to OD_{600} 0.002 and incubated at 37°C with shaking for 18 hours before results were interpreted. The concentration of 13 in antibiotic supplemented media at which there was no observable growth was considered the minimum resensitizing concentration (MRC). For amoxicillin/clavulanic acid, the initial concentration was 8/4 µg mL^{-1}; for cefazolin, 16 µg mL^{-1}. A 50 µL portion of the antibiotic containing media was added to each well of 96-well plates, and 100 µL was added to the top row. A 1.28 µL portion of 5 mg mL^{-1} 13 was added to the top row of each plate to afford a concentration of 64 µg mL^{-1} in the top row of each plate, and 2-fold serial dilutions were performed down the columns. Once the plates were prepared, a day culture of MRSA was diluted to OD_{600} 0.002, and 50 µL was added to each well. The final concentration of MRSA added was OD_{600} 0.001, the final concentration of amoxicillin/clavulanic acid was 4/2 µg mL^{-1}, the final concentration of cefazolin was 8 µg/mL, and the highest concentration of 13 tested was 32 µg mL^{-1}. Plates were incubated overnight at 37 °C with shaking. The MRC value was determined as the concentration of 13 in the presence of antibiotic at which there was no observable overnight growth.

**Mammalian cytotoxicity of 13 in HeLa cells:**

To evaluate the cytotoxicity of 13 in mammalian cells, a cell viability assay was carried out using CellTiter-Glo luminescent cell viability assay kit (Promega). Human cervical adenocarcinoma HeLa cells were seeded on white, cell-culture treated 96-well plates (Corning: 3917) with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, at the densities of 20,000 cells/well. The medium
volume for each well was 100 µL. Cells were incubated at 37 °C in 5% CO₂/95% air for 16 hours. The medium was removed from each well and replaced with 99 µL of warmed fresh medium. To each well was then added 1.0 µL of 13 in DMSO with the final concentrations of 1.56-200 µg/mL. Each concentrate was performed in two replicates. After incubation at 37 °C for another 24 hours, the plates were equilibrated to room temperature for 30 minutes. 100 µL of CellTiter-Glo reagent (Promega) was added to each well and mixed for 2 minutes on an orbital shaker. The plate was incubated at room temperature for another 10 minutes to stabilize luminescent signal. The luminescence of each sample was recorded in an Envision Multilabel Plate Reader (Perkin Elmer).

**Phase I metabolic stability of 13 and Of1 in mouse liver microsomes:**

Phase I metabolic stability was conducted as described previously (Keck et al. 2015; Rais et al. 2012). Briefly, for phase I metabolism, the reaction was carried out with 100 mM potassium phosphate buffer, pH 7.4, in the presence of NADPH regenerating system (1.3 mM NADPH, 3.3 mM glucose 6-phosphate, 3.3 mM MgCl₂, 0.4 U/mL glucose-6-phosphate dehydrogenase, 50 µM sodium citrate). Reaction was initiated by addition of the liver microsomes to the incubation mixture (compound final concentration was 1 µM; 0.2 mg mL⁻¹ microsomes). Samples in triplicate were removed at predetermined time points (0, 30 and 60 min) post incubation and compound disappearance was monitored via LC/MS/MS.

Chromatographic analysis was performed using an Accela™ ultra high-performance system consisting of an analytical pump, and an autosampler coupled with TSQ Vantage mass spectrometer (Thermo Fisher Scientific Inc., Waltham MA). Separation of the analyte from potentially interfering material was achieved at ambient temperature using Agilent Eclipse Plus
column (100 x 2.1mm i.d.) packed with a 1.8 µm C18 stationary phase. The mobile phase used was composed of 0.1% Formic Acid in Acetonitrile and 0.1% Formic Acid in H₂O with gradient elution. The total run time for each analyte was 5.0 min. The selected reaction monitoring transitions (SRM) for 13 were 482.054>184.117, 279.051; for Of1 were 543.796>203.855, 308.031.

Phase I metabolism results:

Figure S1. Phase I metabolic stability of 13 and Of1 in mouse liver microsomes. Compared to Of1 compound 13 demonstrated substantial metabolic stability in liver microsomes from mice. Negative controls (data not shown) without cofactors were stable with >90% remaining at 60 min, for both compounds suggesting CYP dependent metabolism. Testosterone used as positive control with <1% remaining at 30 minutes.
Aqueous solubility of 13 and Of1 at pH 7.4:

Solubility studies were conducted as described previously (Shukla et al. 2012). Briefly, compound in excess was placed in 500 µL DPBS and incubated at 37º C while shaking for approximately 48 hours. The saturated solutions were then filtered with a 0.22 micron Millipore filter. Calibration curves for 13 and Of1 were generated in 50% DPBS in acetonitrile, using losartan as an internal standard. Analysis was performed using LC/MS/MS.

Chromatographic analysis was performed using an Accela™ ultra high-performance system consisting of an analytical pump, and an autosampler coupled with TSQ Vantage mass spectrometer (Thermo Fisher Scientific Inc., Waltham MA). Separation of the analyte from potentially interfering material was achieved at ambient temperature using Agilent Eclipse Plus column (100 x 2.1mm i.d.) packed with a 1.8 µm C18 stationary phase. The mobile phase used was composed of 0.1% Formic Acid in Acetonitrile and 0.1% Formic Acid in H₂O with gradient elution. Calibration curves for 13 and Of1 were constructed from the peak area ratio of the analyte to the internal standard with a weighting factor of 1/(nominal concentration) over the 50,000-10 nM and 10,000-10 nM Of1, respectively. Correlation coefficient of greater than 0.99 was obtained in all analytical runs for both analyte. The selected reaction monitoring transitions (SRM) for 13 were 482.054>184.117, 279.051; for Of1 were 543.796>203.855, 308.031.

Solubility results:

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<th>Compound</th>
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<tr>
<td>13</td>
<td>587 ± 108</td>
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<tr>
<td>Of1</td>
<td>0.85 ± 0.30</td>
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Table S1. Saturation solubility of 13 compared to Of1

Procedure for measuring membrane permeability:

Permeability of 13 and Of1 were performed in MDCK cells as previously described with minor modifications (Volpe 2011). Briefly, MDCK cells were seeded at a density of 800,000 cells/well (1.12 cm²) on Corning® Transwell polycarbonate membrane inserts (pore size 0.4 µm, membrane diam. 12 mm). All studies were conducted with HBSS supplemented with 10 mM HEPES (pH 7.4). The integrity of cell monolayers was evaluated by measuring [14C]-mannitol permeability across the monolayers and by measuring TEER values using EVOM2 epithelial voltohmometer (World Precision Instruments). 13 (10 µM) and Of1 (1 µM) were incubated at the apical (A) side of the membrane and aliquots of the buffer in basolateral (B) chambers were removed for the determination of the drug concentration. Metoprolol was used as a high permeability marker. Drug concentrations were determined by LC-MS/MS (as described previously) and A>B permeability coefficient were calculated.

Permeability results:

The permeability of 13 was low and calculated to be 2.11E-06 ± 3.53E-07 cm s⁻¹. For (low permeability marker) mannitol was 1.51E-06 ± 2.67E-07 cm s⁻¹ and for (high permeability marker) metoprolol 5.35E-05± 6.05E-06 cm s⁻¹. (Note: Of1 was not quantifiable due to low solubility and concentrations in the receiver were below the limit of quantification.)

Procedure for single-dose pharmacokinetic studies:
Female CD-1 mice (Charles River Laboratories, CA) at the ages of 6-7 weeks each received a single i.p. dose at 30 mg kg$^{-1}$ of 13. Blood was collected from the treated mice at 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6, 8 and 24 hr post-dose for processing to plasma. All samples were analyzed by LC-MS/MS for drug levels.
A.2 Nuclear Magnetic Resonance Spectra
Chapter 2
Chapter 3
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![Chemical structure](image)

![NMR spectrum](image)
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![Diagram of chemical structure and NMR spectrum](image-url)