Synthesis and Evaluation of Acridine and Acridone Based Compound as Anti-Cancer and Anti-Bacterial Agents.

A dissertation

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Abstract

After the discovery of acridine in the late 1800's, the acridine class of molecules attracted much synthetic interest due to their usefulness as dyestuffs and the discovery that certain acridine derivatives possessed anti-bacterial properties. It was this discovery that lead aminoacridines to be used heavily as anti-bacterial agents until they were supplanted by the use of sulfonamides and the discovery of the penicillin's. Despite falling out of favor as anti-bacterial agents, acridine derivatives were still used in the treatment of other diseases such as malaria and cancer.

Despite falling out of use as anti-bacterial agents, one area where the acridine class of compounds may find a new use is in the treatment of drug-resistant bacterial infections, namely MRSA. Infections caused by numerous drug-resistant bacteria have become a major health issue in the world today which has led to a pressing need for the development of novel agents to treat these infections.

We report within this thesis the synthesis and evaluation of numerous acridine and acridone based compounds that possess activity against a number of strains of MRSA. In an attempt to elucidate a potential mechanism of action, the active compounds were also tested in DNA intercalation assays and screens against other known bacterial targets. Despite our best efforts, the mechanism of these novel acridine compounds was not elucidated and is hypothesized to be an interaction with the bacterial cell membrane.

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Chapter 1: Synthetic Methods to Access Acridines

Bernthsen Acridine Synthesis:

Acridine, first isolated in 1870 from coal tar,[1] has attracted significant synthetic attention over the years. In 1884, Bernthsen reported the first synthesis of acridine in which diphenylamine was condensed with benzoic acid using zinc chloride and high temperatures (**Figure 1**).[2]

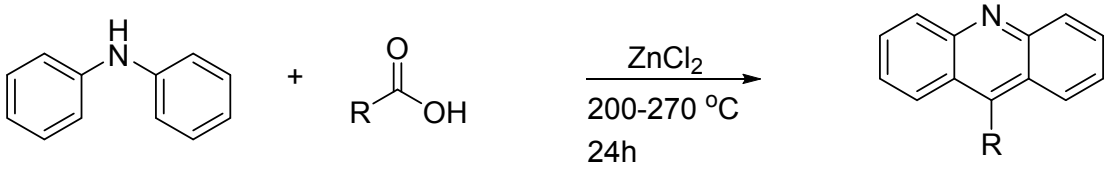


Figure 1: General scheme of the Bernthsen Acridine Synthesis

Since this first synthesis of acridine by Bernthsen, what has come to be known as the Bernthsen Acridine Synthesis has been used to synthesize numerous 9-substituted acridine analogs by varying the carboxylic acid coupling partner.

Due to the fact that the original Bernthsen synthesis often suffered from low yields and the need for each set of reaction conditions to be dependent on the substrate,[3] there is much room for improvement on the reaction to provide better yields with less variation of conditions between substrates. One major improvement of the Bernthsen synthesis came in 1961 when Popp reported the use of polyphosphoric acid (PPA) as both the solvent and acid in the Bernthsen acridine synthesis. In using PPA instead of zinc chloride, Popp found that reaction times could be shortened greatly from 24 hours to 15 minutes (**Figure 2**). It was also found that reaction between electron rich substrates such as *p*-aminobenzoic acid and diphenylamine were successful using Popp's method

while the original synthesis failed to afford any desired acridine. However as with the original synthesis, acridines made from electron deficient substrates such as *p*-nitrobenzoic acid were still not accessible.[4]

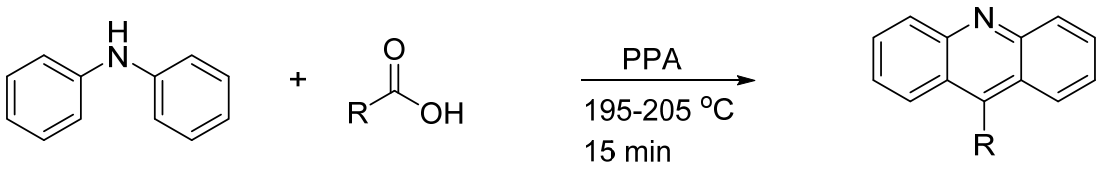


Figure 2: Popp's use of PPA in the Bernthsen Acridine Synthesis

The most recent improvement to the Bernthsen synthesis has been using a microwave reactor instead of the traditional thermal methods used in both the original synthesis and Popp's modification. In 2002 it was reported that using the original zinc chloride conditions (Scheme 3), 9-substituted acridines made from both aromatic and aliphatic carboxylic acids could be made in good yields with short reaction times using microwave assisted synthesis.[5] The newest report in 2011 allowed for the replacement of zinc chloride with *p*-toulenesulfonic acid as the catalyst (**Figure 3**) to afford a wider range of 9-substituted acridines, including ones derived from electron poor carboxylic acids which were previously inaccessible using the Bernthsen reaction.[6]

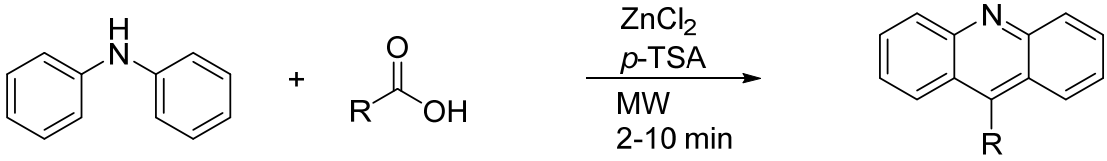


Figure 3: Microwave assisted Bernthsen reactions

Ullmann Synthesis:

While the Bernthsen synthesis has been used to make a large number of acridines, the most common route to synthesizing acridines is via cyclization of *N*-phenylanthranilic acid derivatives.[7] The synthesis of these derivatives was first described in 1885 by Jourdan via a coupling of an aryl halide and an aryl amine. The reaction only proceeded with strongly electron withdrawing groups that activated the aryl amine.[8,9] In 1903, Fritz Ullmann demonstrated that the addition of copper to the coupling reaction allowed for facile coupling of non-activated aryl amines with aryl halides.[10] This discovery lead to the copper catalyzed coupling between aryl halides and aryl amines to be known as the Ullmann reaction (sometimes also called the Ullmann-Jourdan reaction).[7] Shown below in **Figure 4** is the general reaction scheme of the Ullmann reaction.

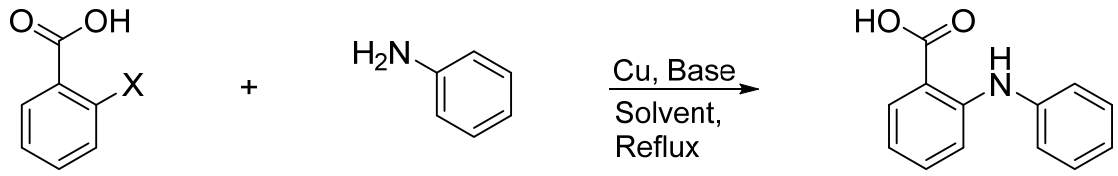


Figure 4: General scheme of the Ullmann reaction.

As mentioned, copper is required as a catalyst for non-activated anilines. At least one equivalent of base is also required as one equivalent of HX (where X can be F, Cl, Br, or I) is liberated in the course of the reaction. There is a great variation on possible solvents with most reactions being run in water,[11] higher boiling alcohols (amyl alcohol being common),[9,7] or DMF.[12] The use of high temperatures, generally greater than 100 °C is required for the reaction to

proceed, though lower temperature variants have been discovered in more recent years.[13]

Though the exact mechanism of the reaction is still not fully known and likely varies between coupling partners and the exact reaction conditions, there has been a large volume of work trying to elucidate the probable mechanisms of the Ullmann reaction. Shown in **Figure 5** one of the first mechanisms proposed is the formation of an aryl copper(III) species that undergoes nucleophilic attack by the aniline.[7,14] This mechanism can also be used to explain some of the difficulties such as the production of side products common with the Ullmann reaction as generally alcohols or water are used which can also undergo nucleophilic attack on the aryl copper(III) intermediate.[7]

a

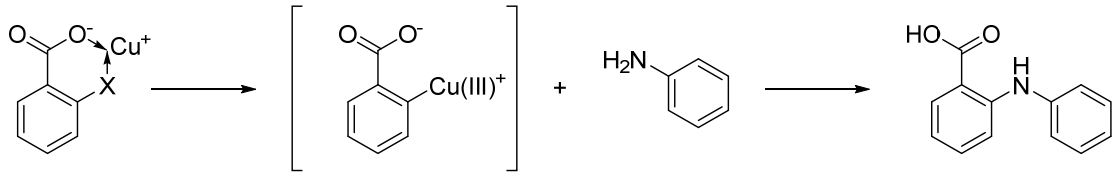


Figure 5: Proposed mechanism of the Ullmann reaction proceeding through a Cu(III) intermediate. Figure adapted from reference 7.

Rather than the oxidative addition of the copper into the aryl-halogen bond happening first to afford the copper(III) intermediate, recent research suggests that a copper-aniline complex actually forms first and this complex is what undergoes oxidative addition to the aryl-halogen bond followed by subsequent reductive elimination to afford the desired *N*-phenylanthranilic acid.[15]

The other major suggested pathway that has garnered a lot of attention has been the possibility of the reaction proceeding through a single electron transfer (SET)

mechanism. The radical mechanism of nucleophilic aromatic substitution reactions first was proposed in 1937 by Waters.[16] Originally the radical mechanism was merely speculation, however in the 1960's, extensive work was done to show that a single electron transfer (SET) mechanism was a plausible pathway.[15]

Work done by Bunnett looking at the amination of 5- or 6- substituted halopesudocumines proposed that if the reaction proceeded through an aryne mechanism, that there should be an equal ratio of the possible products. In the case of the iodopseudocumine, the expected product ratio was vastly shifted which suggested an alternate to the proposed aryne mechanism. To elucidate this possible mechanism, Bunnett used triphenylhydrazine as a radical scavenger and noted the ratio of products was back in line with what is expected from the aryne mechanism. This finding lead to some of the first evidence of a SET mechanism and the designation $S_{RN}1$ (substitution, radical-nucleophilic, unimolecular) for this type of reaction.[17]

Although there was much more research into the SET mechanism, it was not until recently that a computational study by Houk and co-workers provided excellent evidence for the viability of the SET pathway.[18] Further experimental evidence that has helped solidify the SET mechanism as a viable pathway, came in a 2012 study by Peters in which they showed an Ullmann coupling catalyzed by copper and light did in fact proceed through a radical mechanism.[19] With the probable mechanisms of the Ullmann reaction elucidated coupled with vast improvements allowing for milder reaction conditions, the Ullmann reaction for

the formation of C-N bonds to form various substituted *N*-phenylanthranilic acids which can be cyclized to acridines, is still a useful transformation 110 years after its discovery.

Buchwald-Hartwig Amination

Despite recent improvements to the Ullmann reaction to couple aryl halides to aryl amines that can be cyclized to acridines, the reaction still has its drawbacks, especially with coupling partners bearing electron donating substituent's.[20] An efficient coupling reaction for any aryl amine and aryl halide combination, including coupling partners with electron donating groups, was first reported in 1983 by Migita and was catalyzed by palladium in the presence of tin.[21] This reaction was further improved by removing the need for tin and reported on by both Buchwald and Hartwig in 1995 in a reaction that has subsequently become known as the Buchwald-Hartwig amination reaction.[22,23]

The Buchwald-Hartwig amination reaction has become one of the main reactions used in both academia and industry to afford the C-N bond in anilines and anthranilic acids due to its extremely broad substrate scope.[24] This has come through a very large volume of work done optimizing the reaction that has lead to its generality. When it was first reported, the amination reaction had a limited scope and as to which combinations of aryl halide, amine, base, and solvent that would work well. One example is in the first report by Hartwig, aryl iodides performed very poorly in this reaction.[23] This however changed upon the discovery of the second generation of catalysts that used palladium with DPPF

as the ligand. This allowed for near quantitatitive coupling of primary amines to aryl iodides.[25,26]

The mechanism of the reaction occurs via the catalytic cycle shown below in **Figure 6.** The first step of the reaction is the oxidative addition of the aryl halide to the palladium-ligand complex. In the case of the amination reaction, the base deprotinates the nucleophile, which in this case is the amine. The nucleophile adds to the palladium-ligand-aryl complex and a reductive elimination step regenerates the palladium-ligand complex and frees the newly formed C-N coupling product.[27]

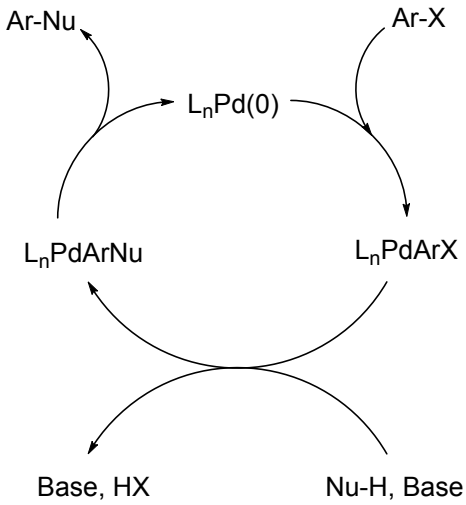


Figure 6: Catalytic cycle of the palladium catalyzed coupling of aryl iodides to amines. Figure adapted from reference 27.

With the Buchwald-Hartwig amination reaction optimized to have a very broad scope of reactivity, there is very little work reported on using the reaction to form N-phenylanthranilic acids that are cyclized to acridines. The one report citing the reaction directly as a vast improvement over the Ullmann reaction was a paper

by Czuk and Raschke that examined a range of electron withdrawing, neutral, and donating coupling partners that were cyclized to acridines using standard methodology.[7] Despite its limited use reported in the literature, the reaction was used successfully by our laboratory to access acridine derivatives that were otherwise problematic to access using the Ullmann reaction.

Chapter 2: Anti-Bacterial Drug Resistance

Methicillin resistant *Staphylococcus aureus* (MRSA):

Supplanting the use of the earliest antibiotics and ushering in what some call the "antibiotic era", the use of penicillin in the early 1940's drastically changed the way we deal with bacterial infections.[28] Along with the ability to now successfully treat routine bacterial infections came what has become a major problem in health care; anti-bacterial drug resistance. Shortly after penicillin became widely used, there were reports of bacteria that were resistant to it.[29] The mechanism of resistance to penicillin was derived from a β-lacatamase enzyme known as penicillinase which cleaved the lactam ring found in penicillin, leading to its inactivation.[30] To combat penicillin resistant strains, a β-lacatamase resistant drug known as methicillin (**Figure 7)** was invented.[31] Much like with penicillin, shortly after its introduction, *S. aureus* developed resistance to methicillin as well giving rise to what is now known as MRSA.[32]

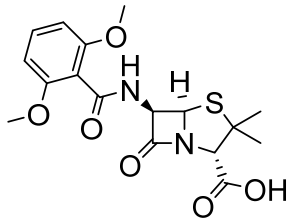


Figure 7: Structure of Methicillin

Unlike the resistance of *S. aureus* to penicillin which stemmed from β-lacatamase enzymes, resistance to methicillin was derived via a novel penicillin binding protein. Shown in **Figure 8,** in methicillin sensitive *S. aureus* (MSSA), the bacteria contains a set of four PBP's that act as transpeptidases that help with peptidoglycan synthesis.[33,34] β-lactam antibiotics such as methicillin work by acetylating the active site serine and preventing these PBP's from acting as transpeptidases.[35]

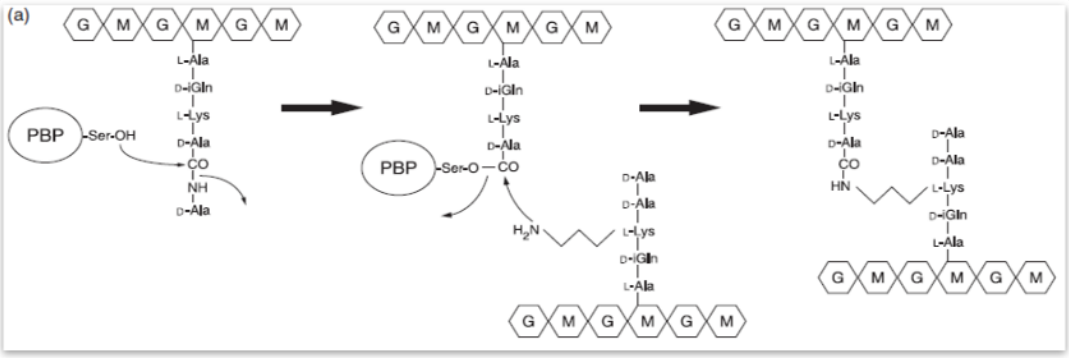


Figure 8: Penicillin-binding protein (PBP) mechanism. Figure adapted from reference 33.

In MRSA, the bacteria acquire a fifth PBP that has a very low affinity for all β-lactam antibiotics. This 78 kDa PBP is known as PBP 2A can have a range of expression and production that can render *S. aureus* somewhat treatable to completely resistant to β-lactam antibiotics.[36,37,38] PBP 2A is encoded for by a gene known as *mecA* which is found on a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*).[39] The size of the SCC*mec* element ranges from 21-67 kb, depending on the variant.[34] As of 2012, there are 11 known variants of the SCC*mec*, all of which contain the *mecA* gene,

which represents nearly a threefold increase in the number of variants from 2004.[31,40]

Ever since the first MRSA infection was discovered in 1961, the incidences of infection by MRSA have skyrocketed across the globe.[41] Originally found mostly in Europe, in the 1980's, MRSA started to become a global epidemic.[31] Due to the emergence of this global epidemic of MRSA infections, both in health care settings and in the community, there have been numerous surveillance programs set up to track MRSA infections. One such program is the SENTRY program which was initiated in 1997 to track the rates of antibacterial resistance over numerous pathogens.[42] Shown in **Figure 9** is some of the original data from the SENTRY program for MRSA. In the United States, MRSA rates as high as 40% of nosocomial infections were noted between 1997 and 1999.[43]

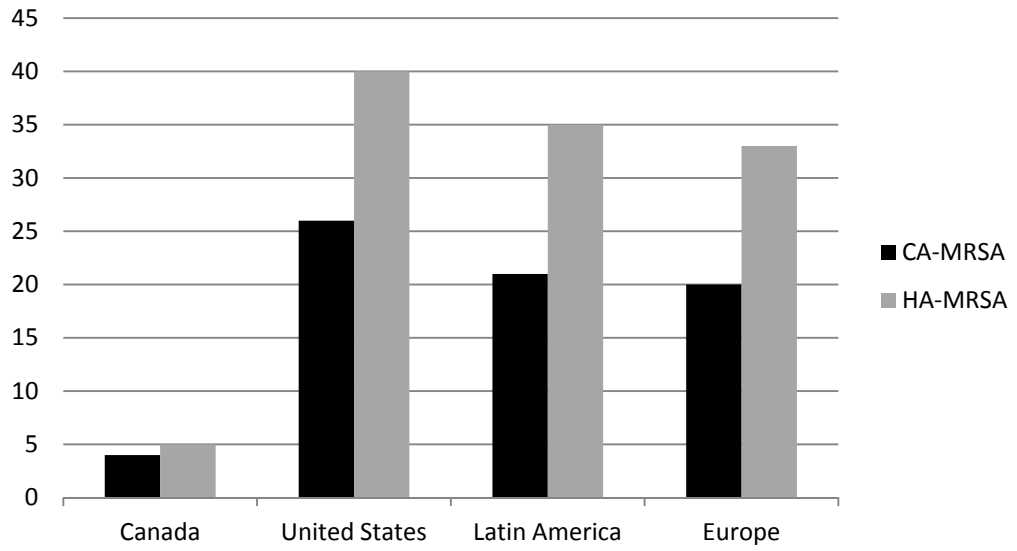


Figure 9: Methicillin resistance rates in different regions around the globe from the SENTRY study between 1997-1999. Figure adapted from reference 43.

In 2003, the MRSA rate peaked at 64% in intensive care units in the United States.[44] In 2005, the MRSA rate for non-nosocomial skin infections peaked at 60%.[45] With the incidences of MRSA rising at an alarming rate, the need to use non β-lactam antibiotics has risen with it. As a result of this increased antibiotic use, some strains of MRSA have grown to be resistant to other classes of antibiotics such as the fluoroquinolones, tetracyclines, and aminoglycocides.[46] For this reason, the gold standard of treatment for MRSA infections has become vancomycin.[32]

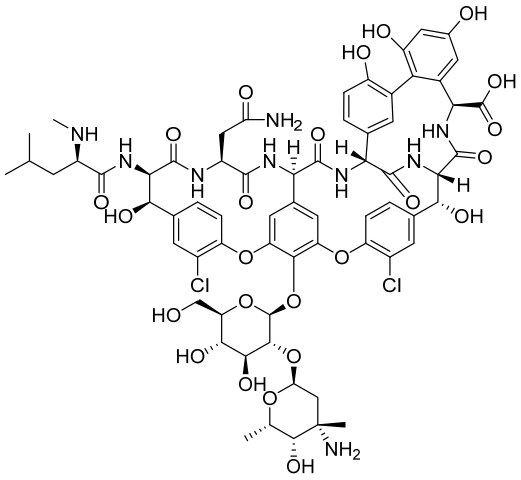


Figure 10: Structure of Vancomycin

Shown in **Figure 10**, vancomycin was first approved for use in 1958 as a treatment for penicillin resistant *S. aureus* infections.[47] Vancomycin is a glycopeptide antibiotic that exerts its antibacterial activity by inhibiting cell wall biosynthesis in a manner independent from the penicillin's.[47,48] Due to advances in the development of novel antibiotics that were effective against *S. aureus* coupled with poor bioavailability and nephrotoxicity, vancomycin quickly fell out of

use and it wasn't until MRSA started becoming resistant to multiple antibiotics that vancomycin found widespread use again.[47,49]

With vancomycin finding widespread use again to combat the most resistant strains of MRSA, given the history of *S. aureus* to develop drug resistance, it was not a big surprise that vancomycin-resistant strains of *S. aureus* started to emerge. Research in the early 1990's showed that *S. aureus* can gain resistance to vancomycin through the transfer of the *VanA* gene of vancomycin resistant *Enterococcus* (VRE).[50] With a major increase in the number of cases of VRE coupled with the ever growing number of cases of MRSA, the first case of vancomycin resistant *S. aureus* (VRSA) was documented in 1997.[51] However this case of VRSA was not due to the presence of the *VanA* gene, it was due to the bacteria growing a thicker cell wall that prevents vancomycin from interacting with its cellular target.[52,53] It was not until 2002 that the first documented case of VRSA containing the *VanA* gene transferred from VRE showed up, proving this gene transfer could occur *in vivo*.[54,55]

With the likelihood of more cases of MRSA becoming resistant to vancomycin, there are a number of other antibacterial agents that are either approved or in trials currently. Shown in **Figure 11** are four of those agents. Ceftaroline is a cephalosporin with broad-spectrum activity that has been approved for use in skin infections caused by MRSA in the United States.[56,57] Tigecycline is a glycylcycline antibiotic that is bacteriostatic and approved in the United states and Europe for skin infections, intra-abdominal infections, and community-acquired pneumonia.[56,58] Trimethoprim is a dihydrofolate reducatase (DHFR)

inhibitor that is used in cases of milder skin infections caused by MRSA.[56,59] Linezolid is a oxazolidinone antibiotic used in pneumonia and bacteremia caused by MRSA.[56,60] Not shown in the figure are telavancin which is a lipoglycopeptide antibiotic that is approved in the United States and used only in severe cases due to its high toxicity.[56,61] Daptomycin is a lipopeptide approved in Europe for skin infections, however it is not effective in cases of pneumonia caused by MRSA.[56,62]

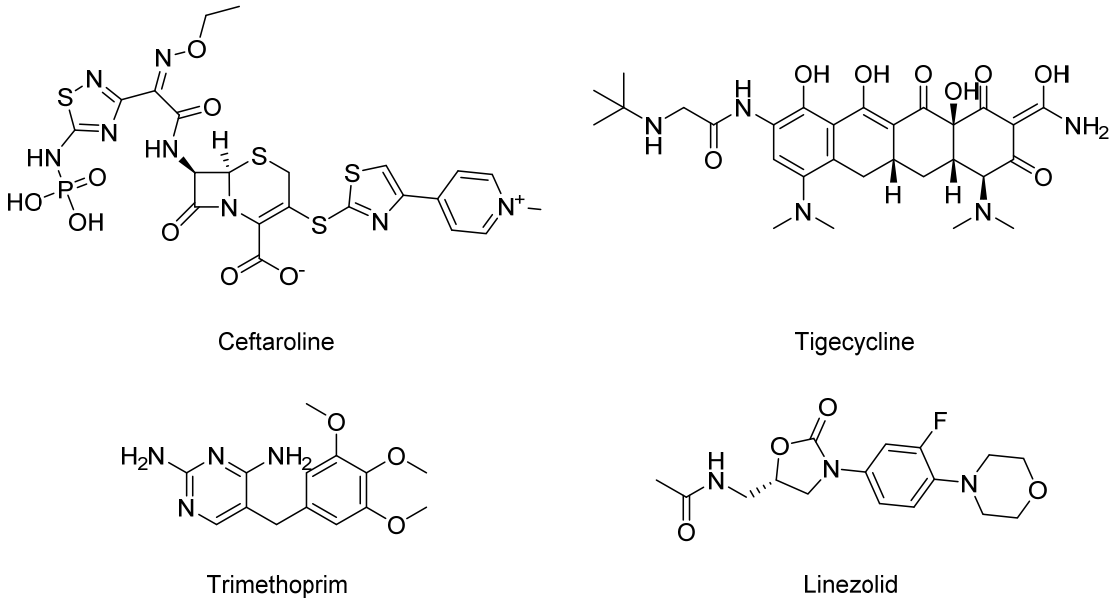


Figure 11: Other drugs used to treat MRSA. Ceftaroline (cephalosporin), tigecycline (glycylcycline), trimethoprim (DHFR inhibitor), and linezolid (oxazolidinone).

Vancomycin Resistant *Enterococcus* (VRE)

The first infection caused by Vancomycin Resistant *Enterococcus* (VRE) was first reported in 1986 in a hospital in England.[63] Only 5 years after the first reported case in England, VRE was reported in New York City hospitals.[64] By the year 2000, the CDC was reporting that 26% of all *enterococcus* infections in intensive care units were resistant to vancomycin.[65] For blood stream infections, the number caused by VRE skyrocketed to almost 81% in 2010.[66] Normally, *Enterococci* are benign inhabitants of the gut flora that only cause infections in cases where there is a confounding illness. *Enterococci* infections are caused primarily by two species, *E. faecium* and *E. faecalis*.[67] Out of these two species, vancomycin resistant strains are far more prevalent in *E. faecium* than in *E. faecalis*.[68]

Resistance of *Enterococci* to vancomycin is derived from modifications of the peptidoglycan synthetic pathway in the bacterial cell wall. Unlike in MRSA, resistance is not derived from a novel protein that has low affinity for the drugs but is derived from modification of the pieces that comprise the cell wall itself.[69] In normal cell wall biosynthesis, a dipeptide comprised of D-Ala-D-Ala is part of the pathway and is also the target for vancomycin and other glycopeptide antibiotics.[66] Resistant strains have several different *Van* genes including; *Van*A, *Van*B, and *VanD* that encode for a depsipeptide D-Ala-D-Lactate instead of the native D-Ala-D-Ala.[70] By changing to D-Ala-D-Lac, a key hydrogen bond is removed in the interaction between the target and vancomycin which leads to a 1000-fold decrease in affinity which causes the resistance to vancomycin.[71]

With an increase in incidents of infections by VRE that are also resistant to β-lactam antibiotics, there have only been two new therapies approved for the treatment of VRE infections in the early 2000s.[66]

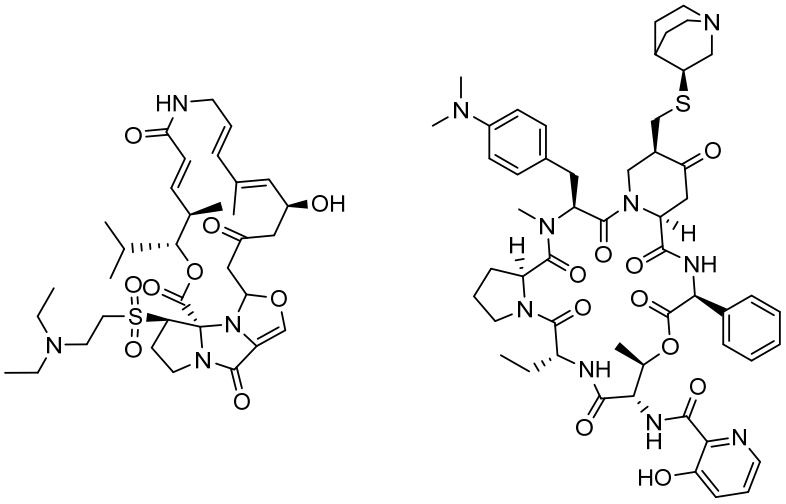


Figure 12: Structures of Dalfopristin (left) and Quinupristin (right)

Dalfopristin-Quinupristin (**Figure 12**) is a mixture of the two drugs belonging to the streptogramin class in a 70:30 ratio respectively.[72] The major drawback of this treatment and the main reason why it is no longer used, despite good activity against VRE, is the fact that it requires a central IV line to administer.[66] The other recently approved drug is linezolid. With excellent oral bioavailability and broad spectrum activity against most gram-positive bacteria, including VRE, it has become the front line antibacterial drug for the treatment of VRE.[73]

Acinetobacter Baumannii (*A. Baumannii*)

Acinetobacter Baumannii is a gram-negative, non-fermentative coccobacillus that is responsible for a wide range of human infections.[74] Drug resistant *A. baumannii* was first discovered in the 1970's. While *A. baumannii* infections are normally associated with health care settings, there have been some reports of community associated infections starting in the early 2000's.[75] Multi-drug resistant *A. baumannii* is resistant to any combination of carbapenems, cephalosporins, ampicillin-sulbactam, aminoglycocides, and fluoroquinolones.[76]

Unlike MRSA and VRE, which derive their resistance through a small set of specific enzymes or mutations, multi-drug resistant *A. baumannii* has a wide array of resistant mechanisms at its disposal for each class of antibiotic drugs. The main resistance mechanism used against β-lactams are β-lacatamase and cephalosporinases that enzymatically degrade the drugs before they can acetylate their respective targets.[77] To a lesser degree, β-lactam resistance is also conferred via a lower membrane permeability in *A. baumannii* than in other micro-organisms.[78]

Resistance to aminoglycocides is caused by two separate mechanisms, the first is enzymatic inactivation of the drug and the second and less prominent is efflux pumps.[79] Aminoglycocide resistant *A. baumannii* is caused primarily by the expression of acetyletransferases, adenyltransferases, and phosphotransferases that inactivate the drug before it can bind its target.[80]

Fluoroquinolones exert their antibacterial action by binding to either DNA gyrase or bacterial topoisomerase IV.[81] In gram negative bacteria such as *A. baumannii*,

DNA gyrase is the primary target for the fluoroquinolones. DNA gyrase is primarily encoded by two different genes, *gyrA* and *gyrB*. It was found that specific mutations in the *gyrA* gene changed the binding site for the fluoroquinolones which vastly decreased their affinity for DNA gyrase and conferred resistance to them.[82]

With several resistance mechanisms available for the majority of commonly used antibiotic agents, options are becoming limited in cases of multi-drug resistant *A. baumannii.* In cases where there is little to no resistance, the front line treatment for infections caused by *A. baumannii* is members of the carbapenem class of antibiotics.[83] In cases where the infection is caused by a multi-drug resistant strain, practitioners have turned to polymyxin B which is a cationic polypeptide with normally excellent activity against gram-negative bacteria.[83,84] Despite its excellent activity, due to growing use, a strain of polymyxin B resistant *A. baumannii* was isolated in 2001 which is cause for great concern due to the polymyxins being a drug of last resort due to its high toxicity.[84] Tetracyclines and glycylcyclines are another class of antibiotics that are used to treat resistant *A. baumannii* infections.[83] However, as with the polymyxins and other previously discussed classes of antibacterial drugs, resistance to tetracyclines and tigecycline has cropped up in 2003[85] and 2007[86] respectively.

As can be seen by the many mechanisms of resistance not only in *A. baumannii* but in MRSA and VRE as well, that there is a pressing need for novel antibiotics that either bypass current resistance mechanisms or act via novel mechanisms or on novel targets.

Chapter 3: Synthesis and Evaluation of 9-alkylaminoacridines with Activity Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Abstract

Introduction

Staphylococcus aureus is one of the leading causes of bacterial infections in humans with symptoms ranging from simple skin infections to severe necrotizing pneumonia.[87,88] Methicillin-resistant *Staphylococcus aureus* (MRSA) was first discovered in 1961, shortly after the introduction of methicillin.[89] Originally, infection by MRSA was limited to those exposed in health care settings.[90] In 1981, it was first reported that MRSA infections have spread to the community and are currently the leading cause of soft tissue and skin infections today not only in the community but also in the clinic.[91,92]

Vancomycin is currently the first choice of antibiotic to treat MRSA infections.[93] However due to the increased use of vancomycin, a situation described as MIC "creep" has been occurring in which more strains of MRSA are requiring higher doses to successfully treat the infection.[94] This upward trend of MIC's against certain strains of MRSA is leading to the development of moderately to highly vancomycin resistant strains.[56] In cases where vancomycin fails, the next line of treatment generally involves linezolid, ceftaroline, or telavancin.[95,96]

Telavancin is a semi-synthetic derivative of vancomycin.[97] Telavancin has superior activity to vancomycin due to its dual mechanism of action. Telavancin

both inhibits cell wall biosynthesis like traditional glycopeptide antibiotics as well as disrupts the cell membrane by anchoring itself via a lipophilic side chain.[61] Despite the advances in treating MRSA with telavancin, there still remains a major need to develop newer and more effective antibiotics to treat ever evolving drug resistant bacteria.

One potential source of novel antibacterial agents goes back to some of the original antibacterial agents to be used, the aminoacridines. Acridines, originally only used as dyes, were tested in the late 1800's to early 1900's and found to have antibacterial activity. Originally used extensively for the treatment of wounds during the First World War, the acridines fell out of favor until they were re-examined for use in wounds during the Second World War. This led to the widespread use of Aminacrine (9-aminoacridine) in wounds where other treatments had failed.[98] The use of acridines as antibacterial agents occurred up until penicillin became more widely available.[99]

Research by Albert and Goldacre showed that acridines that exist primarily as cations at physiological pH are the best antibacterial agents. Further research into the structural features that gave acridines and other heterocyclic bases antibacterial properties also showed that a large planar surface area is also a key feature, as highly ionized aminoquinolines and aminopyridines did not show any activity in antibacterial assays.[100] Knowing the flat planar surface area was a key feature, it was determined in 1963 that acridines act on the level of the DNA via intercalating between the base pairs.[101] It was shown that the intercalation of the

acridine into the DNA disrupts the action of DNA topoisomerases, leading to cell death.[102]

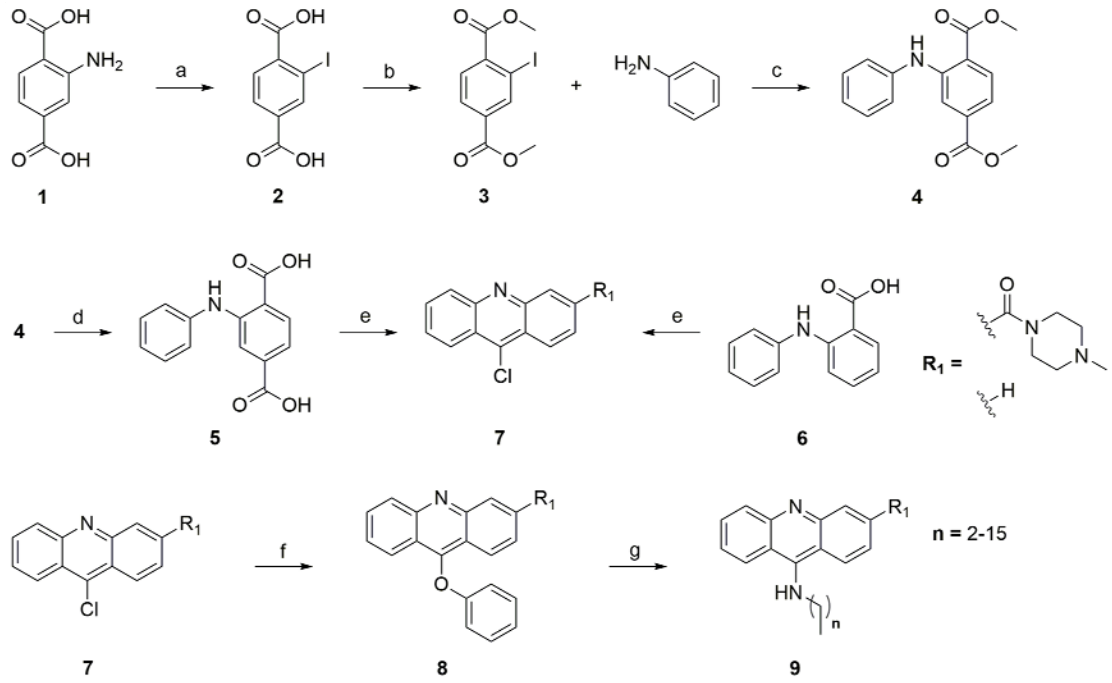
Herein we report the synthesis and evaluation of three series of acridine and acridone based compounds bearing an alkylamino moiety of varying length. To fully probe the effect of the 9-amino substituent, two series of acridines with a 9-aklyamino moiety ranging from three to sixteen carbons were synthesized. As a non-intercalative control, a series of acridones bearing the same alkylamine moieties was synthesized. All compounds were tested in two strains of MRSA and one strain of methicillin-sensitive *Staphylococcus aureus* (MSSA). All of the acridine based compounds were also tested against the DU145 prostate cancer cell line as a general measure of cytotoxicity. In the case of acridines, an active and non-active compound along with the analogous acridones was tested in a DNA intercalation assay as well to explore their mechanism of action.

Synthesis

Two different routes were used to make 2-phenylaminoterephthalic acid **5**. The first route followed a previous published literature procedure.[103] Due to variable yields which were often low using the modified Ullmann-Goldberg coupling, a second and higher yielding route was used to provide the intermediate for the 3-carboxamide substituted acridines. Shown in **Scheme 1** is the second route which provided a route with consistently high yields of the desired coupling product **5**. 2-iodoterephthalic acid **2** was synthesized from 2-aminoterephthalic acid **1** via a Sandmeyer reaction.[104] Dimethyl-2-iodoterephthalate **3** was

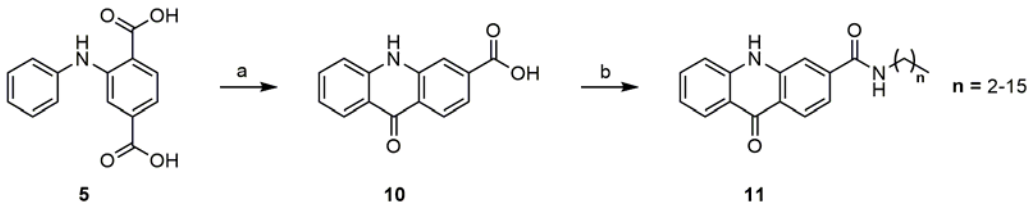
synthesized by reacting **2** with methyl iodide in DMF using potassium carbonate as the base. Dimethyl-2-iodoterephthalate was coupled with aniline using a Buchwald-Hartwig amination reaction with subsequent saponification of the esters with sodium hydroxide to afford 2-phenylaminoterephthalic acid **5**.[7]

Cyclization to give the 9-chloroacridines was accomplished by refluxing **5** in phosphorous oxychloride. In the case of the acridines with no substitution at the 3-position, commercially available 2-phenylanthranillic acid **6** was used instead. The unstable 9-chloro derivatives were converted to stable 9-phenoxy derivatives by reacting with excess phenol. To complete the synthesis of the two series of 9-aminoacridines, the respective 9-phenoxyacridne was reacted with the appropriate alkylamine to yield the desired 9-alkylamino acridines.



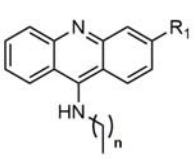
Scheme 1: Synthesis of 9-aminoacridine compounds. Reagents and conditions: (a) i. $NaNO_2$, ii. KI, 1:1 HCl:H_2O, rt; (b) MeI, K_2CO_3, DMF, rt.; (c) $Pd(OAc)_2$, dppf, Cs_2CO_3, toluene, 95° C; (d) 5% NaOH, acetone, rt; (e) (i) $POCl_3$, reflux, (ii) Amine, DCM, rt; (f) phenol, 80° C; (g) amine, phenol, 50-100° C.

The control series of 3-carboxamide acridones was synthesized following the route outlined in **Scheme 2**. **5** was cyclized in polyphosphoric acid to give the acridone acid **10**. BOP-Cl and triethylamine was used to couple **10** with each respective amine in DMF to afford the 3-carboxamide acridone series **11**.[105]

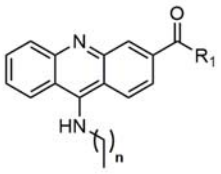


Scheme 2: Synthesis of 3-carboxamide acridone compounds. Reagents and conditions: (a) PPA, 145° C; (b) amine, BOP-Cl, Et_3N, DMF, rt.

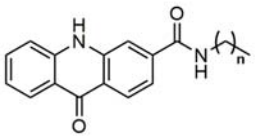
Results and Discussion



Compounds 9a-9n



Compounds 9o-9bb



Compounds 11a-11n

Compound	R_1	n	ATCC 43300	IRDL 6169	IRDL 8545	DU145
9a	H	2	>100	>100	>100	4.76 ± 0.35
9b	H	3	>100	>100	>100	5.01 ± 1.00
9c	H	4	>100	>100	>100	4.55 ± 0.46
9d	H	5	50.58	46.88	>100	5.43 ± 0.67
9e	H	6	24.49	22.86	27.54	5.53 ± 0.83
9f	H	7	8.54	10.79	10.26	5.80 ± 1.17
9g	H	8	3.19	3.59	5.95	6.55 ± 0.78
9h	H	9	2.19	3.29	2.61	6.46 ± 0.28
9i	H	10	2.01	2.73	3.81	6.12 ± 0.32
9j	H	11	2.88	2.73	2.88	5.90 ± 0.62
9k	H	12	3.04	2.80	2.61	5.41 ± 0.75
9l	H	13	3.07	2.15	2.98	5.15 ± 0.41
9m	H	14	6.10	3.21	5.69	4.88 ± 0.64
9n	H	15	24.72	10.14	26.24	4.81 ± 0.74
9o	N-methylpiperazinyl	2	>100	>100	>100	>200
9p	N-methylpiperazinyl	3	>100	>100	>100	83.81 ± 6.90
9q	N-methylpiperazinyl	4	>100	>100	>100	101.18 ± 57.00
9r	N-methylpiperazinyl	5	>100	>100	>100	28.48 ± 2.62
9s	N-methylpiperazinyl	6	>100	>100	>100	19.13 ± 6.76
9t	N-methylpiperazinyl	7	>100	>100	>100	14.22 ± 3.68
9u	N-methylpiperazinyl	8	22.70	23.66	35.48	6.42 ± 1.29
9v	N-methylpiperazinyl	9	12.16	10.74	11.97	5.23 ± 0.54
9w	N-methylpiperazinyl	10	3.61	6.14	6.23	2.52 ± 0.82
9x	N-methylpiperazinyl	11	6.28	5.87	4.80	2.04 ± 1.28
9y	N-methylpiperazinyl	12	2.91	3.07	3.57	2.72 ± 0.62
9z	N-methylpiperazinyl	13	5.82	6.13	6.25	2.76 ± 0.28
9aa	N-methylpiperazinyl	14	4.59	8.59	5.91	3.50 ± 0.91
9bb	N-methylpiperazinyl	15	6.23	9.06	11.59	3.52 ± 1.38
11a-n	-	2-15	>100	>100	>100	ND

Table 1: Anti-bacterial assay results for MRSA (ATCC43300 and IRDL6169) and MSSA (IRDL8545) and prostate cancer cytotoxicity. Anti-bacterial data is MIC99 (μM), anti-cancer data is EC50 (μM).

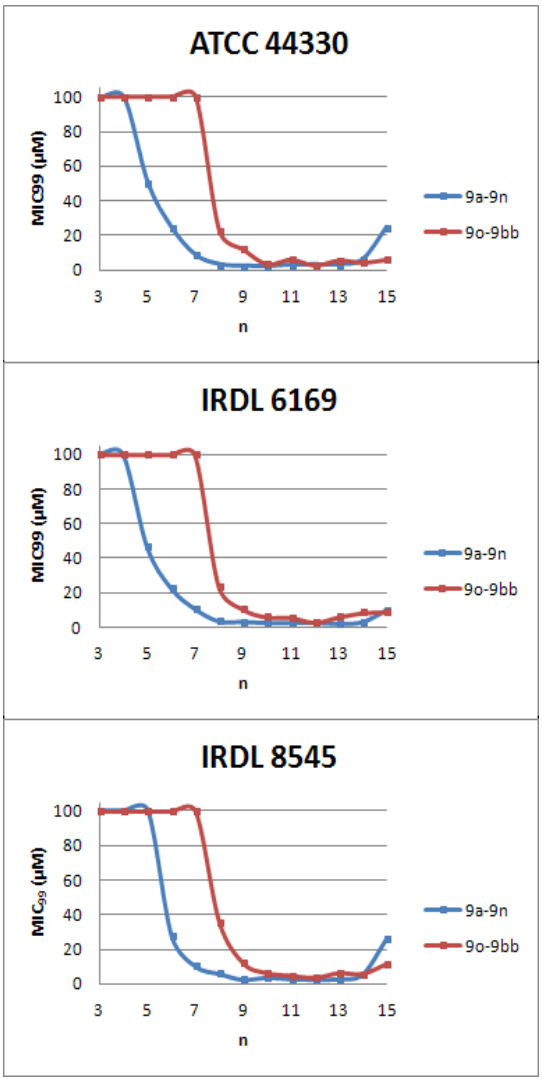


Figure 13: MIC vs. alkylamino length for the three cell lines tested.

Anti-bacterial activities (MIC_{99}) and for the two series of acridines and one series of acridones is reported in **Table 1**. Compounds were tested in two strains of MRSA and one strain of MSSA. In the two series of acridines, both showed antibacterial activity to be tied to the length of the 9-aklylamino substituent. Shown in Table 1, the un-substituted acridines 19a-19n, antibacterial activity was first exhibited when the 9-amino substituent reached six carbons and reached max potency when the 9-amino substituent reaches ten carbons with no further increase in activity. In the case of the 3-carboxamide substituted acridines 19o-19bb, antibacterial activity was first exhibited when the 9-amino substituent reached nine carbons and reached peak activity at thirteen carbons with no increase in activity after that length. In the case of both the un-substituted and substituted acridines, the max potency did not differ significantly (graphically represented in **Figure 13**). In each series, there was no discernible difference in activity between the resistant and sensitive

S. aureus, which suggests that there is no selectivity in these compounds for resistant strains.

Also shown in **Table 1** is the cytotoxicity data for the two series of acridines in the DU145 prostate cancer cell line. In the case of the un-substituted acridines (9a-9n) cytotoxicity was not linked to the length of the alkylamino substituent like it was in the case of the anti-bacterial activities. These compounds all exhibited a relatively high degree of cytotoxicity across the entire series (~5 μM) whereas the 3-carboxamide substituted acridines cytotoxicity depended on the length of the 9-alkylamino substituent in a nearly identical trend as the anti-bacterial activity.

As a control, we also synthesized a series of acridones bearing the alkylamine attached via a 3-carboxamide linker. Shown in **Table 1** is the anti-bacterial activity for the series of acridones, 11a-11n. None of the acridones tested showed any activity at 100 μM. This result was as expected given the acridones were designed to be neutral at physiological pH and previous testing in our laboratory on similar compounds showed that 3-carboxamide substituted acridones are poor DNA intercalators.[103] Lacking both of these features that the 9-alkylamino acridines contain, along with lacking any anti-bacterial activity, this is strong evidence that the anti-bacterial activity of the acridines is derived from a combination of both carbon chain length and the ability to exist as a cation under physiological conditions rather than just by the length of the carbon chain of the substituent.

Given our data shows that the antibacterial activity of the acridine compounds is driven by the length of the 9-amino substituent, we tested both an active and inactive compound from each series along with analogous acridone controls in a DNA intercalation assay to determine if these compounds are acting via multiple mechanisms. Shown in **Figure 14** are the DNA intercalation assays for the six compounds tested. In this assay, relaxed DNA becomes supercoiled when a compound intercalates into DNA.[106] Surprisingly, compounds 9a and 9o, corresponding to the two propyl analogs which show no anti-bacterial activity show the highest degree of intercalation. Compounds 9h and 9v, which correspond to the active decyl compounds, show very weak intercalation. As expected from previous work on similar compounds, neither compound 11a or 11h showed that the intercalated DNA.

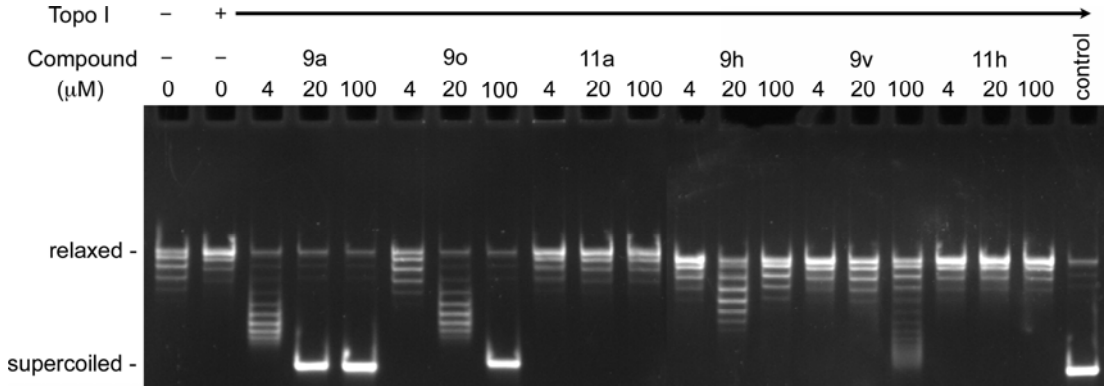


Figure 14: DNA intercalation assay.

Conclusions

This study has examined two series of 9-alkylamino acridines and a series of 3-carboxamide acridones. In the case of both the un-substituted and 3-carboxamide substituted acridines, anti-bacterial activity increased with the length of the alkylamino substituent at the 9 position at different rates but reaching similar peak values. None of the 3-carboxamide substituted acridones showed any anti-bacterial activity in the cell lines tested.

In an attempt to elucidate the mechanism of action of the acridine compounds, a DNA intercalation assay was run as acridines are well known to intercalate into DNA and interfere with replication.[101,107,108] Seen in **Figure 13** is the data for the intercalation assay in which contrary to expected, the non active acridines showed the highest degree of intercalation. Some of the most active compound (9h and 9v) show very low levels of intercalation, even at high concentration. This is somewhat surprising though not unprecedented. In a previous study, Galy and co-workers showed that increasing the length of an alkylamine at the 9 position of an acridine increased its affinity for DNA until the length reaches 6 carbons long. After that length, any increase in additional length led to a decrease in affinity for DNA.[108] Given our compounds started showing activity around the length of alkylamino substituent that lead to a decrease in DNA intercalation, this led us to look into an alternative mechanism of action for our compounds.

Since the most active acridines are likely not acting through a mechanism that involves DNA intercalation, we propose that they are instead killing the bacteria by membrane disruption. The bacterial cell membrane contains a higher percentage of negative charges with allows for compounds that contain a positive charge to interact with them.[109,110] This ionic interaction and subsequent membrane disruption is what gives quaternary ammonium compounds,[111] cationic anti-microbial peptides,[112] and cationic amphiphiles[109] their anti-bacterial properties. Given 9-aminoacridnes have a pKa around 9-10 they would be protinated at physiological pH.[98,113] Shown in **Figure 15** are the two possible tautomers of a 9-alkylamino acridine. Given the longer and more hydrophobic alkyls are the most active, we believe these acridines are acting in a similar fashion as cationic amphiphiles, disrupting the bacterial cell membrane, and leading to bacterial death. This mechanism could be advantageous as it is does not hit a specific target, meaning it could be harder for the bacteria to develop new resistance to these compounds via modification of the target.

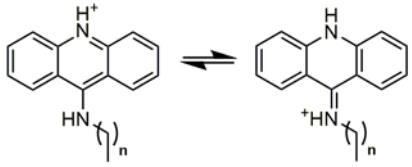


Figure 15: Two possible tautomers of the protinated acridine.

Despite the good anti-bacterial activity of the most active compounds, the most active compounds also showed high levels of cytotoxicity, which would hinder their use. Future work on these compounds would involve performing membrane disruption assays to confirm the mechanism of action. Also, fine tuning the properties of these 9-alkylamino acridines to allow them to maintain their anti-

bacterial activity while cutting down on their cytotoxicity could lead to new anti-bacterial compounds to treat up and coming drug resistant bacteria.

Experimental

Anti-bacterial assay (broth micro dilution)

Compounds were tested against Methicillin-resistant *Staphylococcus aureus* (MRSA) purchased from the American Type Culture Collection (ATCC): ATCC 43300. Two clinical isolates of *S. aureus* (MRSA: IDRL 6169 and MSSA: IDRL 8545) were also tested. *S. aureus* was grown in BBL™ Trypticase™ Soy Broth (BD) at 37 °C.

Microbial susceptibility testing was performed using an adaptation of the standard broth micro dilution assay.[114] Briefly, bacteria were grown to mid-log phase, diluted with fresh medium to an optical density at 600 nm (OD_{600}) of 0.030-0.060 and then diluted again 1:10. This suspension (195 µL) was added to wells in a 96 well microtiter plate (Sarstedt) and 5 µL of compound dissolved in DMSO was added to give a final concentration of 100 – 0.1 µM at 2.5% DMSO by volume. A DMSO negative control and standard antibiotic positive controls were included in each plate. All compounds were tested in triplicate for each concentration. Plates were sealed with parafilm, placed in a Ziploc bag to prevent evaporation, and incubated at 37 °C for 16-20 hours. The OD_{600} values for each well were determined with a plate reader (Biotek, EL800) and the data were standardized to the DMSO control wells. Initial single concentrations were tested

at 100 μM and active compounds were further tested with at least nine concentrations for a full dose response. Dose response curves were generated using GraphPad Prism 4 software and used to determine the MIC_{99} concentrations (minimal concentration that inhibits 99% of growth).

DNA intercalation assay

A DNA intercalation assay was performed as described previously.[115] Briefly, reaction mixtures (20 μl) containing 50 mM Tris-HCl (pH 7.5 at 23°C), 2.5 mM $MgCl_2$, 0.5 mM DTT, 50 μg/ml BSA, 300 ng of relaxed plasmid DNA, and 2 units of human topoisomerase I (Topogen, Port Orange, Fl) were incubated at 37 °C for 10 min in the absence or presence of the indicated concentrations of the various compounds. Reactions were terminated by extraction with phenol/chloroform/isoamyl alcohol (25:24:1, v/v) and the DNA products were analyzed by electrophoresis through vertical 1.2% SeaKem ME agarose (Lonza, Rockland, ME) gels (14 x 10 x 0.3 cm) at 2 V/cm for 15 h in TAE buffer [50 mM Tris-HCl (pH 7.9 at 23 ^{o}C), 40 mM sodium acetate and 1 mM EDTA]. Gels were stained with ethidium bromide and photographed using a Stratagene Eagle Eye gel documentation system (Agilent Technologies, Santa Clara, CA).

Cell Titer Blue cytotoxicity assay

DU145 cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (pen/strep). After reaching confluency, cells were treated with trypsin, pelletized, and re-suspended in

growth media. Cell concentration was determined via counting on a hemocytometer and then cells were plated at a concentration of 5000 cells per well and left to incubate for 24 hours. After 24 hours, growth media was removed and the cells were treated with fresh growth media containing 2% DMSO and the compounds starting at 100 μM with a 2-fold dilution factor over 8 wells in triplicate. The treated cells were incubated for 48 hours then viability was tested using the Cell Titer-Blue® assay. Dose response curves and EC_{50} values were calculated using GraphPad Prism 4.0. The assays were repeated on three consecutive days.

Chemistry General

2-iodoterephthalic acid (2): This compound was prepared from 2-aminoterephthalic acid via a Sandmeyer reaction according to the conditions previously published.[104] 74% yield.

Dimethyl-2-iodoterephthalate (3): 2-iodoterephthalic acid (5.56 mmol) was dissolved in DMF and 2.2 equivalents of K_2CO_3 (12.23 mmol) were added and the reaction was stirred at room temperature for 30 min. After 30 min, 2.2 equivalents of methyl iodide (12.23 mmol) were added drop wise and the reaction was stirred another 1 hour at room temperature. The reaction mixture was taken into 600 mL of Et_2O and washed 3x 350 mL of water to remove salts and DMF. Concentration of the organic layer yielded 16.24g (5.07 mmol) of yellow solid. 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H), 3.96 (s, 3H), 7.80 (d, *J* = 8.2 Hz, 1H), 8.04 (dd, *J* = 8.1, 1.7 Hz, 1H), 8.62 (d, *J* = 1.2 Hz, 1H).

Dimethyl-(2-phenylamino)terephthalate (4): Compound was prepared by a modified procedure found in the literature.[7] $Pd(OAc)_2$ (3 mol%), dpff (6 mol%) and aniline (60 mmol) were dissolved in 125 mL dry toluene and stirred under argon at 95° C for 15 min. After 15 min, dimethyl-2-iodoterephthalate (50 mmol) and Cs_2CO_3 (70 mmol) were added. The reaction mixture was stirred at 95 °C for 10 hours. After cooling, the reaction was filtered and concentrated in vacuo. The resultant oil was adsorbed on celite and purified on silica eluting 10% EtOAc:Hexanes to 20% EtOAc:Hexanes to afford 13.5g (47.32 mmol) of yellow solid. 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 3.93 (s, 3H), 7.13 (m, 1H), 7.25 (m, 2H), 7.35 (m, 3H), 7.91, (s, 1H), 8.01 (d, *J*= 8.4 Hz, 1H), 9.5 (s, 1H).

2-aminoterephthalic acid (5): Dimethyl-(2-phenylamino)terephthalate (47.32 mmol) was dissolved in 500 mL of acetone. 350 mL of 5% NaOH was added and the reaction was stirred at room temperature overnight. The reaction was then neutralized with 3N HCl and the organics were removed under reduced pressure. The resultant yellow solid was collected via filtration and washed 2x with 150mL water to yield 10.9g (42.37 mmol) of yellow solid. 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (m, 1H), 7.28 (m, 3H), 7.40 (m, 2H), 7.74 (s, 1H), 7.98 (d, *J*= 8.2 Hz, 1H), 9.61 (s, 1H), 13.25 (br, s, 2H); HRMS ($C_{14}H_{12}N_2$).

General Procedure for 9-chloroacridine (7) and 9-phenoxyacridine (8): Previously published procedures were followed for the synthesis of these compounds.[103]

General Procedure for Compounds 9a-9n:

9-propylamino-acridine (9a). 9-phenoxy-acridine (0.5 mmol) was added to a round bottomed flask with 1.0 g of phenol. Propylamine (1.0 mmol) was added and the reaction was stirred at 100 °C for 1 hour. Upon cooling, the reaction was diluted in CH_2Cl_2 and purified on silica eluting 90:10 CH_2Cl_2:MeOH to 85:10:5 CH_2Cl_2:MeOH:Et_3N. The fractions containing the product were concentrated and placed under high vacuum where the product solidified as a yellow solid. Yield: 31%; ^{1}H NMR (600 MHz, CD_3OD) δ 1.08 (t, *J* = 7.3 Hz, 3H), 2.015 (sextet, *J* = 7.3 Hz, 2H), 4.12 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.9 Hz, 2H), 8.50 (d, *J* = 8.5, 2H); HRMS ($C_{16}H_{17}N_2$) $[M+H]^+$: found *m/z* 237.1392, calcd 237.1386.

9-butylamino-acridine (9b). Yield: 22%; ^{1}H NMR (600 MHz, CD_3OD) δ 1.01 (t, *J* = 7.3 Hz, 3H), 1.515 (sextet, *J* = 7.3 Hz, 2H), 1.97 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.55 (t, *J* = 7.9 Hz, 2H), 7.805 (d, *J* = 8.5 Hz, 2H), 7.95 (t, *J* = 7.9 Hz, 2H), 8.495 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{17}H_{19}N_2$) $[M+H]^+$: found *m/z* 251.1547, calcd 251.1543.

9-pentylamino-acridine (9c). Yield: 28%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.39-1.49 (m, 4H), 1.99 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.9, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.9 Hz, 2H), 8.50 (d, *J* = 8.5 Hz, 2H); HRMS ($C_{18}H_{21}N_2$) $[M+H]^+$: found *m/z* 265.1702, calcd 265.1699.

9-hexylamino-acridine (9d). Yield: 72%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.3 Hz, 3H), 1.31-1.39 (m, 4H), 1.47 (m, 2H), 1.97 (pentet, *J* = 7.6 Hz, 2H), 4.12 (t, *J* = 7.3 Hz, 2H), 7.54 (t, *J* = 7.9 Hz, 2H), 7.795 (d, *J* = 8.2 Hz, 2H), 7.93 (t, *J* = 7.9 Hz, 2H), 8.47 (d, *J* = 8.5 Hz, 2H); HRMS ($C_{19}H_{23}N_2$) $[M+H]^+$: found *m/z* 279.1859, calcd 279.1856.

9-heptylamino-acridine (9e). Yield: 19%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 6.5 Hz, 3H), 1.30-1.31 (m, 4H), 1.38-1.40 (m, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.6 Hz, 2H), 8.50 (d, *J* = 8.5 Hz, 2H); HRMS ($C_{20}H_{25}N_2$) $[M+H]^+$: found *m/z* 293.2006, calcd 293.2012.

9-octylamino-acridine (9f). Yield: 18%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, 7.0 Hz, 3H), 1.22-1.40 (m, 8H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.9 Hz, 2H), 8.495 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{21}H_{27}N_2$) $[M+H]^+$: found *m/z* 307.2161, calcd 307.2169.

9-nonylamino-acridine (9g). Yield: 51%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.0 Hz, 3H), 1.22-1.33 (m, 8H), 1.35-1.40 (m, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 7.55 (t, *J* = 8.2 Hz, 2H), 7.815 (d, *J* = 8.5 Hz, 2H), 7.95 (t, *J* = 8.2 Hz, 2H), 8.49 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{22}H_{29}N_2$) $[M+H]^+$: found *m/z* 321.2322, calcd 321.2325.

9-decylamino-acridine (9h). Yield: 33%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.3 Hz, 3H), 1.21-1.34 (br, m, 10H), 1.38 (pentet, *J* = 7.6 Hz, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.9 Hz, 2H), 8.50 (d, *J* = 8.5 Hz, 2H); HRMS ($C_{23}H_{31}N_2$) $[M+H]^+$: found *m/z* 335.2482, calcd 335.2482.

9-undecylamino-acridine (9i). Yield: 37%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.3 Hz, 3H), 1.21-1.34 (br, m, 12H), 1.34-1.41 (m, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.9 Hz, 2H), 8.50 (d, *J* = 8.5 Hz, 2H); HRMS ($C_{24}H_{33}N_2$) $[M+H]^+$: found *m/z* 349.2629, calcd 349.2638.

9-dodecylamino-acridine (9j). Yield: 20%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.3 Hz, 3H), 1.22-1.34 (br, m, 14H), 1.38 (pentet, *J* = 7.6 Hz, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 7.55 (t, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.95 (t, *J* = 7.3 Hz, 2H), 8.495 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{25}H_{35}N_2$) $[M+H]^+$: found *m/z* 363.2785, calcd 363.2795.

9-tridecylamino-acridine (9k). Yield: 48%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.22-1.34 (br, m, 16H), 1.34-1.41 (m, 2H), 1.48 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.57 (t, *J* = 7.3 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.6 Hz, 2H), 8.50 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{26}H_{37}N_2$) $[M+H]^+$: found *m/z* 377.2937, calcd 377.2951.

9-tetradecylamino-acridine (9l). Yield: 28%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.21-1.34 (br, m, 18H), 1.34-1.41 (m, 2H), 1.43-1.51 (m, 2H), 1.98 (pentet, *J* = 7.6 Hz, 2H), 4.14 (t, *J* = 7.0 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.95 (t, *J* = 7.6 Hz, 2H), 8.495 (d, *J* = 8.5, 2H); HRMS ($C_{27}H_{39}N_2$) $[M+H]^+$: found *m/z* 391.3096, calcd 391.3108.

9-pentadecylamino-acridine (9m). Yield: 67%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.3 Hz, 3H), 1.22-1.34 (br, m, 20H), 1.38 (pentet, *J* = 7.3 Hz, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.3 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.95 (t, *J* = 7.9 Hz, 2H), 8.495 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{28}H_{41}N_2$) $[M+H]^+$: found *m/z* 405.3247, calcd 405.3264.

9-hexadecylamino-acridine (9n). Yield: 35%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.22-1.34 (br, m, 22H), 1.38 (pentet, *J* = 7.0 Hz, 2H), 1.47 (pentet, *J* = 7.6 Hz, 2H), 1.98 (pentet, *J* = 7.3 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.96 (t, *J* = 7.3 Hz, 2H), 8.495 (d, *J* = 8.8 Hz, 2H); HRMS ($C_{29}H_{43}N_2$) $[M+H]^+$: found *m/z* 419.3407, calcd 419.3421.

General Procedure for Compounds 9o-9bb:

3-(4-Methylpiperazine-1-carbonyl)-9-propylamino-acridine) (9o). 3-(4-Methylpiperazine-1-carbonyl)-9-phenoxy-acridine (0.25 mmol) was added to a round bottomed flask with 1.0 g of phenol. Propylamine (0.5 mmol) was then

added and the reaction mixture was stirred at 100 °C for 1 hour. Upon cooling, the reaction was diluted in CH_2Cl_2 and purified on silica eluting 90:10 CH_2Cl_2:MeOH to 85:10:5 CH_2Cl_2:MeOH:Et_3N. The fractions containing the product were concentrated and placed under high vacuum where the product solidified as a yellow-orange solid. Yield: 66%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.98 (t, *J* = 7.6 Hz, 3H), 1.84 (sextet, *J* = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.57 (br, s, 2H), 3.56 (br, s, 2H), 3.84 (br, s, 2H), 3.88 (t, *J* = 7.3 Hz, 2H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.86-7.90 (m, 2H), 8.325 (d, *J* = 8.8 Hz, 1H), 8.41 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{22}H_{27}N_4O$) $[M+H]^+$: found *m/z* 263.2190, calcd 363.2179.

3-(4-Methylpiperazine-1-carbonyl)-9-butylamino-acridine) (9p). Yield: 22%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.93 (t, *J* = 7.3 Hz, 3H), 1.43 (sextet, *J* = 7.3 Hz, 2H), 1.81 (pentet, *J* = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.58 (br, s, 2H), 3.56 (br, s, 2H), 3.85 (br, s, 2H), 3.93 (t, *J* = 7.0 Hz, 2H), 7.335 (dd, J_a = 9.1 Hz, J_b = 1.8 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.86-7.89 (m, 2H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.42 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{23}H_{29}N_4O$) $[M+H]^+$: found *m/z* 377.2344, calcd 377.2336.

3-(4-Methylpiperazine-1-carbonyl)-9-pentylamino-acridine) (9q). Yield: 36%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.91 (t, *J* = 7.0 Hz, 3H), 1.36-1.44 (m, 4H), 1.91 (pentet, *J* = 7.6 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.58 (br, s, 2H), 3.53 (br, s, 2H), 3.85 (br, s, 2H), 4.03 (t, *J* = 7.3 Hz, 2H), 7.425 (dd, J_a = 9.1 Hz,

J_b = 1.8 Hz, 1H), 7.48 (m, 1H), 7.84 (m, 3H), 8.41 (d, J = 8.8 Hz, 1H), 8.495 (d, J = 9.1 Hz, 1H); HRMS ($C_{24}H_{31}N_4O$) $[M+H]^+$: found *m/z* 391.2503, calcd 391.2492.

3-(4-Methylpiperazine-1-carbonyl)-9-hexylamino-acridine) (9r). Yield: 59%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.84 (t, J = 7.0 Hz, 3H), 1.26-1.30 (m, 4H), 1.38-.142 (m, 2H), 1.83 (pentet, J = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.58 (br, s, 2H), 3.56 (br, s, 2H), 3.85 (br, s, 2H), 3.93 (t, J = 7.0 Hz, 2H), 7.34 (dd, J_a = 8.8 Hz, J_b = 1.5 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.73 (t, J = 7.9 Hz, 1H), 7.86-7.89 (m, 2H), 8.335 (d, J = 8.8 Hz, 1H), 8.425 (d, J = 8.8 Hz, 1H); HRMS ($C_{25}H_{33}N_4O$) $[M+H]^+$: found *m/z* 405.2649, calcd 405.2649.

3-(4-Methylpiperazine-1-carbonyl)-9-heptylamino-acridine) (9s). Yield: 48%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.85 (t, J = 7.0 Hz, 3H), 1.21-1.28 (m, 4H), 1.29-1.37 (m, 2H), 1.37-1.43 (m, 2H), 1.86 (pentet, J = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.58 (br, s, 2H), 3.55 (br, s, 2H), 3.85 (br, s, 2H), 3.98 (t, J = 7.3 Hz, 2H), 7.37 (dd, J_a = 8.8 Hz, J_b = 1.5 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 7.77 (t, J = 7.6 Hz, 1H), 7.84-7.87 (m, 2H), 8.365 (d, J = 8.8 Hz, 1H), 8.45 (d, J = 9.1 Hz, 1H); HRMS ($C_{26}H_{35}N_4O$) $[M+H]^+$: found *m/z* 419.2796, calcd 419.2792.

3-(4-Methylpiperazine-1-carbonyl)-9-octylamino-acridine) (9t). Yield: 37%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.85 (t, J = 7.0 Hz, 3H), 1.18-1.30 (m, 8H), 1.40 (pentet, J = 7.6 Hz, 2H), 1.84 (pentet, J = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.58 (br, s, 2H), 3.56 (br, s, 2H), 3.85 (br, s, 2H), 3.96 (t, J = 7.0 Hz, 2H), 7.355 (dd, J_a = 9.1 Hz, J_b = 1.8 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.74 (t, J = 7.9

Hz, 1H), 7.87-7.90 (m, 2H), 8.355 (d, *J* = 8.5 Hz, 1H), 8.44 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{27}H_{37}N_4O$) $[M+H]^+$: found *m/z* 433.2965, calcd 433.2962.

3-(4-Methylpiperazine-1-carbonyl)-9-nonylamino-acridine) (9u). Yield: 45%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.17-1.32 (m, 10H), 1.36-1.42 (m, 2H), 1.84 (pentet, *J* = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.58 (br, s, 2H), 3.56 (br, s, 2H), 3.85 (br, s, 2H), 3.95 (t, *J* = 7.0 Hz, 2H), 7.35 (d, *J* = 8.8 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.74 (t, *J* = 7.3 Hz, 1H), 7.86-7.90 (m, 2H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.435 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{28}H_{39}N_4O$) $[M+H]^+$: found *m/z* 447.3116, calcd 447.3118.

3-(4-Methylpiperazine-1-carbonyl)-9-decylamino-acridine) (9v). Yield: 52%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.22-1.34 (m, 10H), 1.34-1.40 (m, 2H), 1.47 (pentet, *J* = 7.3 Hz, 2H), 1.96 (pentet, *J* = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.59 (br, s, 2H), 3.51 (br, s, 2H), 3.85 (br, s, 2H), 4.11 (t, *J* = 7.3 Hz, 2H), 7.495 (dd, J_a = 8.8 Hz, J_b = 1.5 Hz, 1H), 7.55 (t, *J* = 7.3 Hz, 1H), 7.79-7.83 (m, 2H), 7.93 (t, *J* = 7.0 Hz, 1H), 8.475 (d, *J* = 8.8 Hz, 1H), 8.545 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{29}H_{41}N_4O$) $[M+H]^+$: found *m/z* 461.3268, calcd 461.3275.

3-(4-Methylpiperazine-1-carbonyl)-9-undecylamino-acridine) (9w). Yield: 25%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.0 Hz, 3H), 1.18-1.32 (m, 14H), 1.38 (pentet, *J* = 7.6 Hz, 2H), 1.83 (pentet, *J* = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.58 (br, s, 2H), 3.56 (br, s, 2H), 3.85 (br, s, 2H), 3.93 (t, *J* = 7.3 Hz, 2H), 7.34 (dd, J_a = 8.8 Hz, J_b = 1.8 Hz, 1H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.73 (t, *J*

= 7.3 Hz, 1H), 7.87-7.89 (m, 2H), 8.335 (d, *J* = 8.2 Hz, 1H), 8.425 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{30}H_{43}N_4O$) $[M+H]^+$: found *m/z* 475.3414, calcd 475.3418.

3-(4-Methylpiperazine-1-carbonyl)-9-dodecylamino-acridine) (9x). Yield: 24%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.20-1.36 (m, 16H), 1.43 (pentet, *J* = 7.3 Hz, 2H), 1.90 (pentet, *J* = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.58 (br, s, 2H), 3.53 (br, s, 2H), 3.85 (br, s, 2H), 4.02 (t, *J* = 7.3 Hz, 2H), 7.42 (dd, J_a = 8.8 Hz, J_b = 1.5 Hz, 1H), 7.46-7.49 (m, 1H), 7.81-7.85 (m, 3H), 8.40 (d, *J* = 8.8 Hz, 1H), 8.48 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{31}H_{45}N_4O$) $[M+H]^+$: found *m/z* 489.3572, calcd 489.3575.

3-(4-Methylpiperazine-1-carbonyl)-9-tridecylamino-acridine) (9y). Yield: 28%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.16-1.31 (m, 18H), 1.37 (pentet, *J* = 7.6 Hz, 2H), 1.81 (pentet, *J* = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.57 (br, s, 2H), 3.55 (br, s, 2H), 3.84 (br, s, 2H), 3.92 (t, *J* = 7.0 Hz, 2H), 7.33 (dd, J_a = 8.8 Hz, J_b = 1.8 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.3 Hz, 1H), 7.86-7.89 (m, 2H), 8.325 (d, *J* = 8.2 Hz, 1H), 8.41 (d, *J* = 9.1 Hz, 1H); HRMS ($C_{32}H_{47}N_4O$) $[M+H]^+$: found *m/z* 503.3732, calcd 503.3744.

3-(4-Methylpiperazine-1-carbonyl)-9-tetradecylamino-acridine) (9z). Yield: 19%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.23-1.35 (m, 18H), 1.38 (pentet, *J* = 7.6 Hz, 2H), 1.48 (pentet, *J* = 7.3 Hz, 2H), 1.98 (pentet, *J* = 7.3 Hz, 2H), 2.36 (s, 3H), 2.46 (br, s, 2H), 2.60 (br, s, 2H), 3.51 (br, s, 2H), 3.85 (br, s, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 7.515 (d, *J* = 8.8 Hz, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.79-7.83 (m, 2H), 7.96 (t, *J*= 7.6 Hz, 1H), 8.495 (d, *J* = 8.8 Hz, 1H), 8.565

(d, J = 8.8 Hz, 1H); HRMS ($C_{33}H_{49}N_4O$) $[M+H]^+$: found *m/z* 517.3884, calcd 517.3901.

3-(4-Methylpiperazine-1-carbonyl)-9-pentadecylamino-acridine) (19aa). Yield: 23%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, J = 7.0 Hz, 3H), 1.22-1.33 (m, 20H), 1.37 (pentet, J = 7.6 Hz, 2H), 1.46 (pentet, J = 7.6 Hz, 2H), 1.95 (pentet, J = 7.3 Hz, 2H), 2.35 (s, 3H), 2.46 (br, s, 2H), 2.59 (br, s, 2H), 3.51 (br, s, 2H), 3.85 (br, s, 2H), 4.10 (t, J = 7.3 Hz, 2H), 7.49 (dd, J_a = 8.8 Hz, J_b = 1.5 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.79-7.84 (m, 2H), 7.92 (t, J = 7.0 Hz, 1H), 8.465 (d, J = 8.8 Hz, 1H), 8.535 (d, J = 8.8 Hz, 1H); HRMS ($C_{34}H_{51}N_4O$) $[M+H]^+$: found *m/z* 531.4032, calcd 531.4057.

3-(4-Methylpiperazine-1-carbonyl)-9-hexadecylamino-acridine) (9bb). Yield: 26%; ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, J= 7.3 Hz, 3H), 1.19-1.35 (m, 24H), 1.41 (pentet, J = 7.3 Hz, 2H), 1.87 (pentet, J = 7.3 Hz, 2H), 2.34 (s, 3H), 2.45 (br, s, 2H), 2.58 (br, s, 2H), 3.53 (br, s, 2H), 3.84 (br, s, 2H), 3.99 (t, J = 7.3 Hz, 2H), 7.395 (dd, J_a = 8.8 Hz, J_b = 1.2 Hz, 1H), 7.45 (t, J = 7.0 Hz, 1H), 7.77-7.84 (m, 3H), 8.37 (d, J = 8.8 Hz, 1H), 8.455 (d, J = 8.8 Hz, 1H); HRMS ($C_{35}H_{53}N_4O$) $[M+H]^+$: found *m/z* 545.4216, calcd 545.4214.

9-oxo-9,10-dihydroacridine-3-carboxyilic acid (10): 2-phenylaminoterephthalic acid (15.55 mmol) was dissolved in excess polyphosphoric acid and stirred at 145° C for 3 hours. Upon cooling, the mixture was dumped into 600mL of water and a solid precipitated, which was collected via filtration and washed with water. The resultant solid was taken into 1:1 5%

NaOH:MeOH (60 mL) and filtered. The filtrate was taken to pH 5.0 with acetic acid and allowed to precipitate overnight. The precipitate was collected via filtration and washed with water to yield 2.3g of yellow solid (9.62 mmol). Yield: 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (t, *J*= 7.6 Hz, 1H), 7.57 (d, *J*= 8.4 Hz, 1H), 7.75 (m, 2H), 8.19 (s, 1H), 8.24 (dd, J_a= 8.1 Hz, J_b= 1.1 Hz, 1H), 8.28 (d, *J*= 8.4 Hz, 1H), 12.01 (s, 1H); HRMS ($C_{14}H_8NO_3$) $[M-H]^-$: found *m/z* 238.0519, calcd 238.0510.

General Procedure for Compounds 11a-11n:

***N*-propyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11a):** 9-oxo-9,10-dihydroacridine-3-carboxyilic acid (1 mmol), propylamine (1.2 mmol), BOP-Cl (1.2 mmol), and Et_3N (3 mmol) were dissolved in 20mL dry DMF and stirred at room temperature overnight. The reaction mixture was added to 100mL of 5% $NaHCO_3$ and stirred for 1 hour. A solid precipitated and was collected via filtration, washed with water, and recrystallized from MeOH/H_2O to afford 50 mg of yellow/orange solid (0.18 mmol). Yield: 18%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (t, *J*= 7.4 Hz, 3H), 1.58 (m, 2H), 3.27 (m, 2H), 7.28 (t, *J*= 7.4 Hz, 1H), 7.56 (d, *J*= 8.6 Hz, 1H), 7.64 (d, *J*= 7.6 Hz, 1H), 7.76 (t, *J*= 7.1 Hz, 1H), 7.99 (s, 1H), 8.24 (d, *J*= 7.8 Hz, 1H), 8.27 (d, *J*= 8.4 Hz, 1H), 8.71 (t, *J*= 5.2 Hz, 1H), 11.89 (s, 1H); HRMS ($C_{17}H_{17}N_2O_2$) $[M+H]^+$: found *m/z* 281.1294, calcd 281.1285.

***N*-butyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11b):** Yield: 24%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, *J*= 7.24, 3H), 1.37 (sextet, *J*= 7.24, 2H), 1.55 (pentet, *J*= 7.24, 2H), 3.29 (m, 2H), 7.26 (t, *J*= 7.5, 1H), 7.56 (d, *J*=

7.56, 1H), 7.63 (d, *J*= 8.41, 1H), 7.76 (m, 1H), 7.98 (s, 1H), 8.25 (dd, *J*= 8.41, 13.50, 2H), 8.70 (m, 1H), 11.89 (s, 1H); HRMS ($C_{18}H_{19}N_2O_2$) $[M+H]^+$: found *m/z* 295.1444, calcd 295.1441.

***N*-pentyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11c):** Yield: 23%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (m, 3H), 1.33 (m, 4H), 1.56 (m, 2H), 3.28 (m, 2H), 7.25 (t, *J*= 7.4 Hz, 1H), 7.54 (d, *J*= 8.4 HZ, 1H), 7.56 (d, *J*= 8.6 Hz, 1H), 7.72 (m, 1H), 7.99 (s, 1H), 8.20 (d, *J*= 7.8 Hz, 1H), 8.23 (d, *J*= 8.2 Hz, 1H), 8.67 (m, 1H), 11.90 (s, 1H); HRMS ($C_{19}H_{21}N_2O_2$) $[M+H]^+$: found *m/z* 309.1586, calcd 309.1598.

***N*-hexyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11d):** Yield: 9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, *J*= 6.65 Hz, 3H), 1.25-1.40 (m, 6H), 1.55 (m, 2H), 3.29 (m, 2H), 7.26 (t, *J*= 7.5 Hz, 1H), 7.56 (d, *J*= 8.02 Hz, 1H), 7.63 (dd, *J*= 1.37 Hz, 8.41 Hz, 1H), 7.76 (m, 1H), 7.98 (s, 1H), 8.25 (dd, *J*= 8.41 Hz, 13.50 Hz, 2H), 8.70 (m, 1H), 11.89 (s, 1H); HRMS ($C_{20}H_{23}N_2O_2$) $[M+H]^+$: found *m/z* 323.1757, calcd 323.1754.

***N*-heptyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11e):** Yield: 39%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (m, 3H), 1.25-1.40 (m, 8H), 1.55 (m, 2H), 3.29 (q, *J*= 6.26 Hz, 2H), 7.26 (t, *J*= 7.5 Hz, 1H), 7.56 (d, *J*= 8.02 Hz, 1H), 7.63 (dd, *J*= 1.37 Hz, 8.41 Hz, 1H), 7.76 (m, 1H), 7.98 (s, 1H), 8.25 (dd, *J*= 8.41 Hz, 13.50 Hz, 2H), 8.70 (m, 1H), 11.89 (s, 1H); HRMS ($C_{21}H_{25}N_2O_2$) $[M+H]^+$: found *m/z* 337.1911, calcd 337.1911.

***N*-octyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11f):** Yield: 45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (m, 3H), 1.25-1.40 (m, 10H), 1.55 (m, 2H), 3.30 (m, 2H), 7.26 (t, *J*= 7.5 Hz, 1H), 7.56 (d, *J*= 8.02 Hz, 1H), 7.63 (dd, *J*= 1.37 Hz, 8.41 Hz, 1H), 7.76 (m, 1H), 7.98 (s, 1H), 8.25 (dd, *J*= 8.41 Hz, 13.50 Hz, 2H), 8.70 (m, 1H), 11.89 (s, 1H); HRMS ($C_{22}H_{27}N_2O_2$) $[M+H]^+$: found *m/z* 351.2069, calcd 351.2067.

***N*-nonyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11g):** Yield: 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (m, 3H), 1.25-1.40 (m, 12H), 1.55 (m, 2H), 3.30 (q, *J*= 6.26 Hz, 2H), 7.26 (t, *J*= 7.5 Hz, 1H), 7.56 (d, *J*= 8.02 Hz, 1H), 7.63 (dd, *J*= 1.17 Hz, 8.41 Hz, 1H), 7.76 (m, 1H), 7.98 (s, 1H), 8.25 (dd, *J*= 8.41 Hz, 13.50 Hz, 2H), 8.70 (m, 1H), 11.89 (s, 1H); HRMS ($C_{23}H_{29}N_2O_2$) $[M+H]^+$: found *m/z* 365.2226, calcd 365.2224.

***N*-decyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11h):** Yield: 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (t, *J*= 6.3 Hz, 3H), 1.16-1.39 (m, 14H), 1.55 (m, 2H), 3.28 (m, 2H), 7.28 (t, *J*= 7.4 Hz, 1H), 7.56 (d, *J*= 8.2 Hz, 1H), 7.63 (d, *J*= 8.2 Hz, 1H), 7.75 (t, *J*= 7.2 Hz, 1H), 7.99 (s, 1H), 8.24 (d, *J*= 8.2 Hz, 1H), 8.27 (d, *J*= 8.2 Hz, 1H), 8.69 (t, *J*= 5.5 Hz, 1H), 11.89 (s, 1H); HRMS ($C_{24}H_{31}N_2O_2$) $[M+H]^+$: found *m/z* 379.2380, calcd 379.2380.

***N*-undecyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11i):** Yield: 18%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (m, 3H), 1.17-1.37 (m, 16H), 1.55 (m, 2H), 3.28 (m, 2H), 7.27 (t, *J*= 7.4 Hz, 1H), 7.56 (d, *J*= 8.6 Hz, 1H), 7.62 (dd, J_a= 8.3 Hz, J_b= 1.3 Hz, 1H), 7.75 (t, *J*= 7.0 Hz, 1H), 7.99 (s, 1H), 8.22 (m, 1H), 8.26

(d, *J*= 8.4 Hz, 1H), 8.69 (t, *J*= 5.4 Hz, 1H), 11.92 (s, 1H); HRMS ($C_{25}H_{33}N_2O_2$) $[M+H]^+$: found *m/z* 393.2523, calcd 393.2537.

***N*-dodecyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11j):** Yield: 18%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (t, *J*= 6.7 Hz, 3H), 1.14-1.42 (m, 18H), 1.55 (m, 2H), 3.28 (m, 2H), 7.27 (t, *J*= 7.5 Hz, 1H), 7.56 (d, *J*= 8.2 Hz, 1H), 7.62 (dd, J_a= 8.4 Hz, J_b= 1.4 Hz, 1H), 7.74 (m, 1H), 7.99 (s, 1H), 8.23 (dd, J_a= 8.1 Hz, J_b= 1.3 Hz, 1H), 8.26 (d, *J*= 8.4 Hz, 1H), 8.69 (t, *J*= 5.5 Hz, 1H), 11.91 (s, 1H); HRMS ($C_{26}H_{35}N_2O_2$) $[M+H]^+$: found *m/z* 407.2706, calcd 407.2693.

***N*-tridecyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11k):** Yield: 28%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (t, *J*= 6.8 Hz, 3H), 1.14-1.42 (m, 20H), 1.55 (m, 2H), 3.28 (m, 2H), 7.27 (t, *J*= 7.4 Hz, 1H), 7.56 (d, *J*= 8.4 Hz, 1H), 7.62 (dd, J_a= 8.5 Hz, J_b= 1.3 Hz, 1H), 7.75 (m, 1H), 7.99 (s, 1H), 8.23 (m, 1H),), 8.26 (d, *J*= 8.4 Hz, 1H), 8.69 (t, *J*= 5.4 Hz, 1H), 11.91 (s, 1H); HRMS ($C_{27}H_{37}N_2O_2$) $[M+H]^+$: found *m/z* 421.2830, calcd 421.2823.

***N*-tetradecyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11l):** Yield: 38%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (t, *J*= 6.7 Hz, 3H), 1.14-1.42 (m, 22H), 1.55 (m, 2H), 3.28 (m, 2H), 7.27 (m, 1H), 7.56 (d, *J*= 8.2 Hz, 1H), 7.6 (d, *J*= 8.8 Hz, 1H), 7.77 (m, 1H), 7.99 (s, 1H), 8.23 (d, *J*= 8.6 Hz, 1H), 8.26 (d, *J*= 8.2 Hz, 1H), 8.72 (m, 1H), 11.88 (s, 1H); HRMS ($C_{28}H_{39}N_2O_2$) $[M+H]^+$: found *m/z* 435.3014, calcd 435.3013.

***N*-pentadecyl-9-oxo-9,10-dihydroacridine-3-carboxamide (11m):**

Yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (t, *J*= 6.7 Hz, 3H), 1.14-1.42 (m, 24H), 1.55 (m, 2H), 3.28 (m, 2H), 7.26 (m, 1H), 7.55 (d, *J*= 8.8 Hz, 1H), 7.60 (d, *J*= 9.0 Hz, 1H), 7.73, (m, 1H), 7.99 (s, 1H), 8.23 (d, *J*= 8.0 Hz, 1H), 8.26 (d, *J*= 8.6 Hz, 1H), 8.67 (m, 1H), 11.92 (s, 1H); HRMS ($C_{29}H_{41}N_2O_2$) $[M+H]^+$: found *m/z* 449.3183, calcd 449.3163

Chapter 4: Synthesis and Evaluation of Acridine and Acridone Epoxides with Anti-bacterial Activity

Introduction

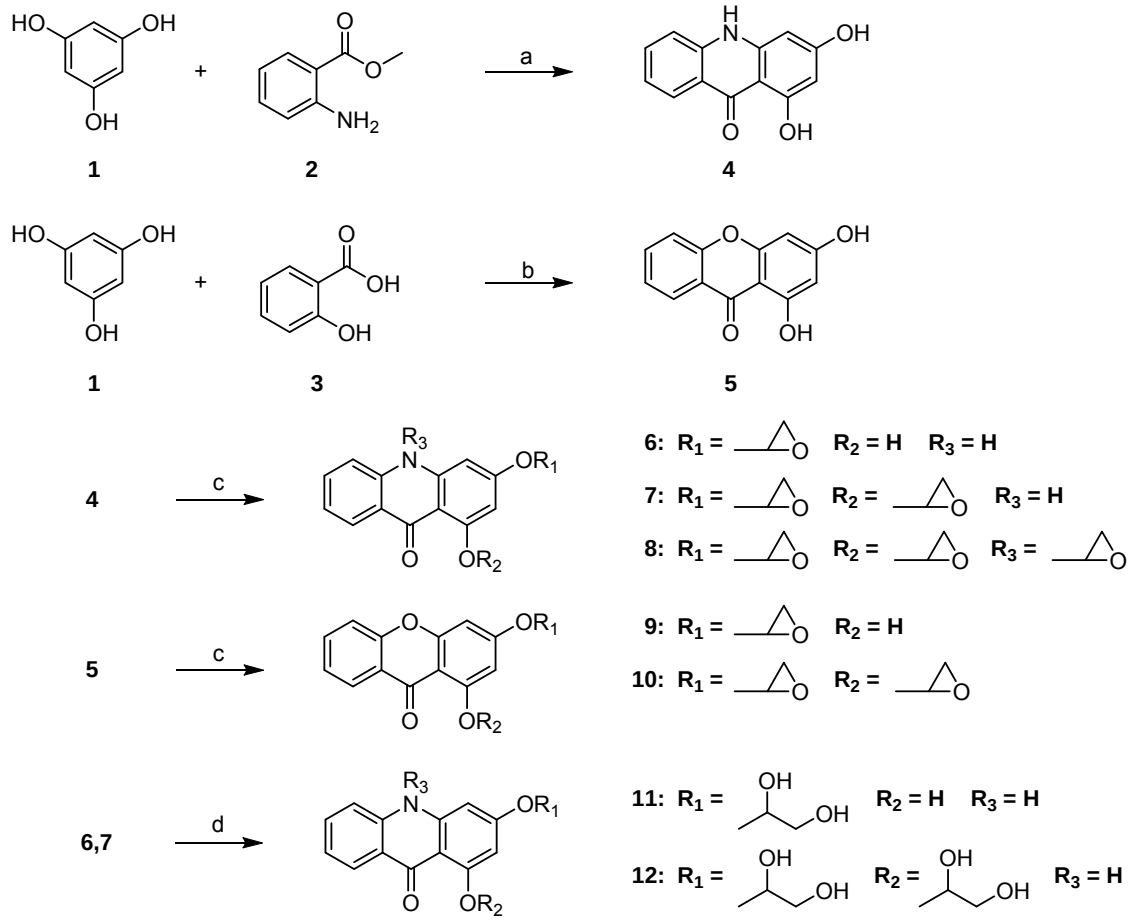
Acridone alkaloids are a diverse class of natural products. The main source of the acridone alkaloids is the Rutaceae family of plants.[116,117] Acridones have been tested in vitro and found to be active against cancers,[118] herpes,[119] psoriasis,[120] and malaria,[121] Despite their broad activity, there has been very little work looking at acridone based compounds as anti-bacterial agents. One recent study looked at a small sample of *N*-alkylated acridone products against both gram positive and negative bacteria. It was found that these simple acridones possess moderate anti-bacterial activity when compared to ciprofloxacin.[122] Another recent study looked at the effects of coupling oxadiazoles to the basic acridone core and found them to posses moderate activity against a small sampling of gram positive and negative bacteria.[123] Outside of these two recent studies, there has not been much reported on the development of acridone based anti-bacterial agents.

With the ever changing landscape of bacterial drug resistance, a pressing need exists for the development of new drugs with either improved activity against known targets or drugs with novel mechanisms of action. One of the most prevalent of these drug resistant bacteria is Methicillin-resistant *Staphylococcus aureus* (MRSA). Known since the 1960's,[89] MRSA has evolved from being mainly isolated to health care settings to being prevalent throughout the

community.[87] In the United States, community associated MRSA is predominantly caused by two strains, USA300 and USA400.[91] Of these two strains, the USA300 strain is the most prevalent and it contains genes for Panton-Valentine leukocidin (PVL) which is a known virulence factor associated with MRSA infections and complications associated with the infection such as bacteremia.[124] While the USA300 strain has taken over as the most prominent strain in the United States, the USA400 strain is still prevalent and contains many of the same virulence factors that the USA300 strain has along with several of the same clinical indications such as necrotizing pneumonia.[125]

Herein we report the synthesis of acridine, acridone, and xanthone compounds bearing 1,2-epoxypropyl substituents at varying positions that show promising activity against both USA300 and USA400 strains of MRSA. Ring opened 1,2-diol analogues were also synthesized as controls to probe the necessity of the epoxide. All compounds that were active against at least one strain of MRSA were subjected to a vero cell cytotoxicity assay as a measure of general toxicity of the compounds. To probe the mechanism of action, a select sampling of active compounds were screened in a whole cell assay in an attempt elucidate mechanism of action.

Synthesis

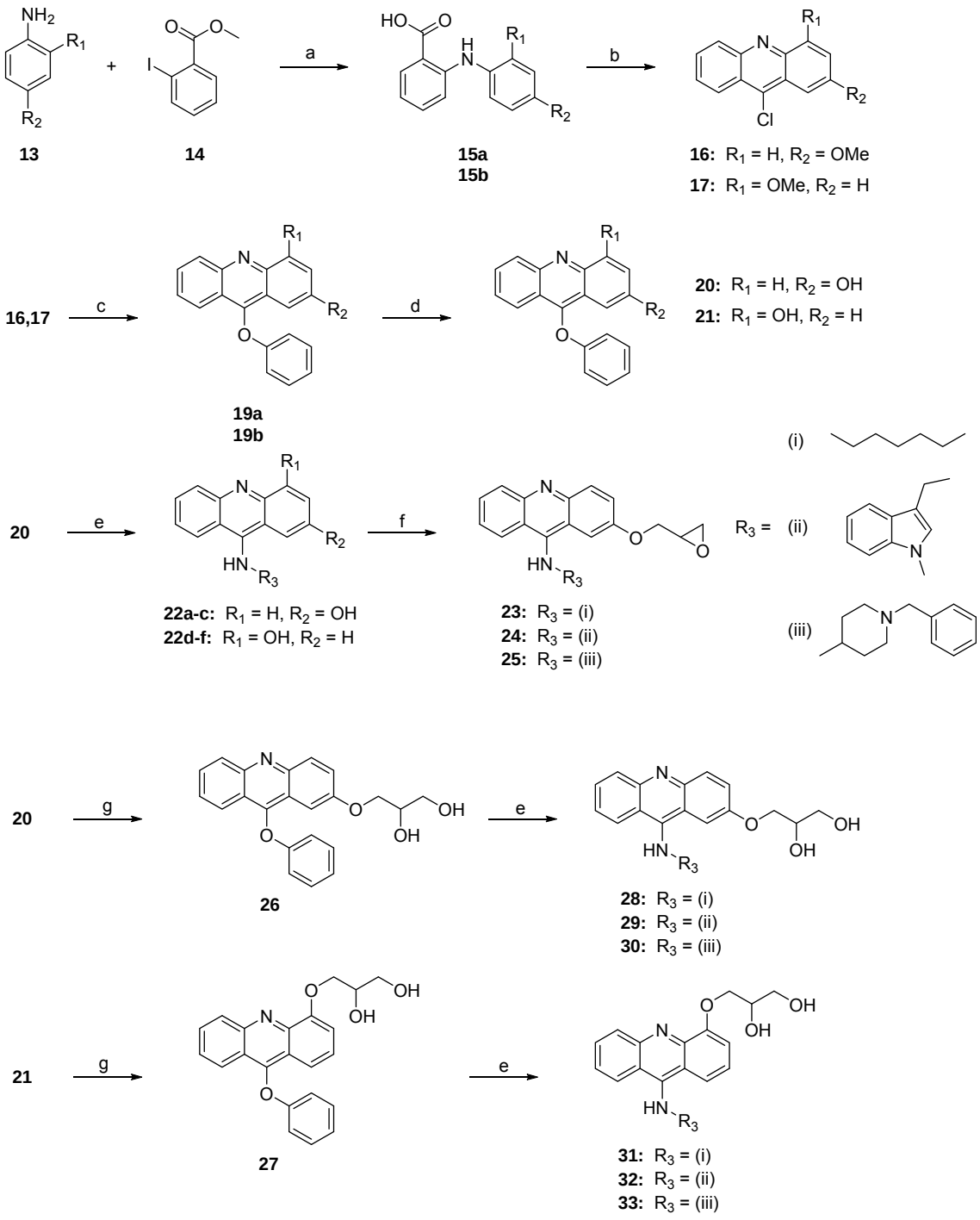


Scheme 3: Synthesis of Acridone and Xanthone Epoxides. Reagents and conditions: (a) PTSA, Hexanol, reflux; (b) $POCl_3$, Zn, 70 °C; (c) Epibromohydrin, Cs_2CO_3, Acetone/DMF, 40-70 °C; (d) 20% NaOH $_{(aq)}$, 70 °C

Shown in **Scheme 3**, synthesis of the acridone based compounds started with a condensation between phloroglucinol **1** and methyl anthranilate **2** to give the 1,3-dihydroxy acridone **4**. Treatment of acridone **4** with cesium carbonate followed by epibromohydrin afforded the desired epoxy acridones **6-8**. By only using a slight excess of epibromohydrin, we were able to isolate both analogs **6** and **7** from one reaction. When we used a large excess of epibromohydrin, we were

able to drive the reaction to alkylate all three positions and isolate **8** as the major product. Hydrolysis of the acridone epoxides **6** and **7** to the respective diols **11-12** was accomplished in good yields by stirring overnight in 20% aqueous sodium hydroxide.

In the case of the xanthone analogs, phloroglucinol **1** was condensed with salicylic acid **3** in phosphorous oxychloride with zinc to afford the 1,3-dihydroxy xanthone **5**. Using the same system as for the acridones in which only a slight excess of epibromohydrin was used, both the mono and di-epoxy xanthones **9-10** were synthesized in one pot.



Scheme 4: Synthesis of acridine analogs. Reagents and conditions: (a) i. $Pd(OAc)_2$, dppf, Cs_2CO_3, toluene, 95 °C, ii. 5% NaOH, acetone, rt; (b) $POCl_3$, 125 °C; (c) Phenol, 80 °C; (d) BBr_3, DCM, rt; (e) amine, phenol, 100 °C; (f) Epibromohydrin, Cs_2CO_3, DMF/MeCN, 70 °C; (g) 3-chloro-1,2-propanediol, Cs_2CO_3, DMF/MeCN, 70 °C

Shown in **Scheme 4**, synthesis of the acridine analogs began with a modified Buchwald-Hartwig amination reaction[7] between methyl-2-iodobenzoate **14** and the appropriate anisidine **13.** We chose to use the Buchwald-Hartwig amination reaction here due to the poor yields and messy reactions given by the traditional Ullmann-Jourdan reaction, especially on our substrates which lack strong electron-withdrawing groups. Hydrolysis of the methyl ester affords carboxylic acids **15a** and **15b**. The choice to use anisidines was due to the instability of phenols towards phosphorous oxychloride which is used to cyclize **15a** and **15b** to the 9-chloroacridines **16** and **17**. 9-chloroacridines **16** and **17** were reacted with molten phenol to give stable 9-phenoxy intermediates **19a** and **19b**. At this stage of the synthesis it was decided, to de-protect the protected phenols to allow for more rapid diversification of the scaffold.

Due to the 9 position of a substituted acridine being amenable to either nucleophilic aromatic substitution or hydrolysis, we attempted to find mild conditions that would cleave the methyl ester to the phenol. Our first attempt at demethylation was to try an aluminum chloride and 1-dodecanethiol system.[126] In our hands, this reaction failed to give any desired hydroxy acridine compound but instead gave a complex mixture of side products. We then tried a sodium methoxide and 1-dodecanethiol system which was reported to be mild and selective for aromatic methyl ethers,[127] however on our system, this reaction failed to afford our desired hydroxy acridine as well and only returned mostly starting material with some minor side products. We ultimately settled on using boron tribromide in dichloromethane at room temperature[128] to afford the desired

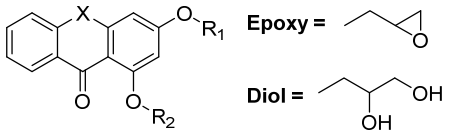
hydroxy acridine intermediates **20** and **21** in moderate yields due to the likely hydrolysis or decomposition of the 9-phenoxyacridine in the presence of small amounts of HBr.

With hydroxy acridines **20** and **21** in hand, treatment of **20** with each respective primary amine in phenol at 100 °C afforded the desired 9-amino-hydroxy acridines **22a-c** in moderate to good yields. Alkylation of each derivative of **22** with epibromohydrin in dimethylformamide/acetonitrile (1:1) using cesium carbonate as a base gave us our desired 9-amino acridine epoxides **23-25**. In the case of the 9-amino-4-hydroxy acridines derived from **21** following the same method used for **22**, we were unsuccessful in obtaining pure 9-amino-4-epoxy acridines. Despite utilizing many different column conditions and both silica and basic alumina, we were never able to separate the unidentified impurity that was co-eluting with our desired compounds.

Our decision to use the N-methylated tryptamine stemmed from our inability to selectively alkylate the phenol on the acridine in the presence of the free indole NH. This is likely due to the similar pKa values of the indole nitrogen (~21)[129] to the phenol (~18)[130] in dimethylsulfoxide (we expect pKa values to be similar in our dimethylformamide solvent system). To accomplish this, we followed a previously published procedure.[131] Using this as the amine instead of tryptamine gave us easy access to our desired analogs in which the epoxide is linked through the phenol on the acridine ring.

Our attempts to use a similar hydrolysis of the epoxide as we did with the acridones failed to yield our desired 1,2-diols. This is due to the sensitivity of 9-amino acridines to hydrolysis to the acridone under basic, aqueous conditions.[132] This meant we had to introduce the diol directly. To allow for a more expedient synthesis of analogs, instead of waiting until the final stage to alkylate the hydroxy acridines **20** and **21**, we used the same dimethylformamide/acetonitrile/cesium carbonate system to alkylate the 9-phenoxy-hydroxy acridine using 3-chloro-1,2-propanediol to afford the 9-phenoxy acridine diols **26** and **27**. Using the same conditions described above for introducing the 9-amino group afforded the desired 9-amino acridine diol compounds **28-33**. Unlike the case of the 4-substituted acridine epoxides, since we alkylated the 4-position prior to installing the amine, we did not have the same purification issue we had with the epoxides, which allowed us to access all of our desired diol analogs.

Results and Discussion

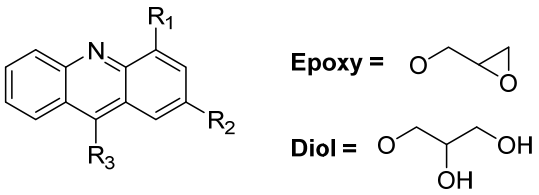


Cpd	X	R_1	R_2	MIC: (μM) USA400 (MW2)	USA300 (1371)	MSSA (MSA553)	Vero Cell Toxicity
4	NH	OH	OH	>100	>100	>100	NT
5	O	OH	OH	>100	>100	>100	NT
6	NH	Epoxy[a]	OH	>100	>100	>100	>100
7	NH	Epoxy	Epoxy	23.6	47.1	23.6	94.3
8	N-Epoxy	Epoxy	Epoxy	80.9	40.5	20.2	>100
9	O	Epoxy	OH	>100	>100	>100	>100
10	O	Epoxy	Epoxy	23.5	23.5	11.8	94.0
11	NH	Diol[b]	OH	>100	>100	>100	NT
12	NH	Diol	Diol	>100	>100	>100	NT

Table 2: Anti-bacterial Data and Vero Cell (kidney epithelial cells) Toxicity (μM). [a]Epoxy denotes 1,2-epoxypropane, [b]Diol denotes 1,2-propanediol, NT = not tested

Shown in **Table 2** are the results for the acridone and xanthone based compounds. In the case of compounds **4** and **5** in which there was no substitution off either the acridone or xanthone ring, the compounds showed no anti-bacterial activity. When an epoxy group was substituted at the 3- position only, acridone **6** showed slight anti-bacterial activity while xanthone **9** still showed no activity. When substituted at both the 1- and 3- position, acridone **7** and xanthone **10** both showed significant anti-bacterial activity against both strains of MRSA and MSSA. Given the increase in activity with the increase in substitution,

we were surprised when the addition of a third epoxy group in compound **8** actually showed a decrease in activity. These compounds also showed moderate toxicity towards vero cells, however there is a 2-4 fold difference between anti-bacterial activity and vero cell toxicity indicating a possibility for a narrow therapeutic window. When we ring opened the two most active acridones to their respective diols, all anti-bacterial activity was lost which is strong evidence that these compounds are exerting their activity via the epoxide, likely through a mechanism that involves either DNA or protein alkylation. In an attempt to elucidate what this possible site of alkylation may be, these compounds were tested in an assay in which a small library of antisense RNA was used to down regulate selected *t*RNA synthetases, the subunits A and B of DNA gyrase, and bacterial topoisomerase IV. Down regulation of these synthetases and enzymes produces strains that are hyper-sensitive to anti-bacterial agents if the compound is acting via the down regulated target.[133,134] In this assay, our most active compounds failed to show an increase in activity in the sensitized strains which suggests these compounds are not acting via a specific target we tested for.



Cpd	R_1	R_2	R3[d]	MIC: (μM) USA400 (MW2)	USA300 (1371)	MSSA (MSA553)	Vero Cell Toxicity
22a	H	OH	8C	6.2	3.1	3.1	49.6
22b	H	OH	4A1BP	83.4	41.7	83.4	>100
22c	H	OH	NMeTryp	>100	21.8	21.8	87.1
22d	OH	H	8C	49.6	24.8	24.8	99.2
22e	OH	H	4A1BP	83.4	41.7	41.7	>100
22f	OH	H	NMeTryp	43.5	43.5	43.5	>100
23	H	Epoxy	8C	10.6	21.1	5.3	42.3
24	H	Epoxy	NMeTryp	75.6	18.9	18.9	75.6
25	H	Epoxy	4A1BP	18.2	18.2	9.1	72.8
28	H	Diol	8C	80.7	10.1	10.1	40.4
29	H	Diol	NMeTryp	>100	>100	>100	>100
30	H	Diol	4A1BP	>100	>100	>100	NT
31	Diol	H	8C	10.1	10.1	10.1	40.4
32	Diol	H	4A1BP	>100	72.5	72.5	>100
33	Diol	H	NMeTryp	>100	69.9	69.9	>100

Table 3: Anti-Bacterial Activity and Vero Cell Toxicity of Acridine Based Compounds (μM). [a]Epoxy denotes 1,2-epoxypropane, [b]Diol denotes 1,2-propanediol, NT = not tested, [d]8C = octylamino, 4A1BP = 4-amino-1-benzyliperidinyl, NMeTryp = 1-methyl-tryptamino

Shown in **Table 3** is our data on the anti-bacterial activity and vero cell toxicity of the acridine based compounds bearing either epoxy or diol substituents. Unlike the acridone and xanthone based compounds, compounds **22a-22f**, lacking either an epoxy or diol substituent showed good to excellent anti-bacterial activity. It is interesting to note that within the acridines, compounds **22a**, **22d**, **23**, **28**, and **31** bearing an octylamino group at the 9-position showed the highest

activity regardless of ring substitution. The vero cell toxicity followed this same trend, which is indicative of these compounds acting through a target that is not specific to bacteria. When comparing the ring opened diols **29** and **30** to their epoxide counterparts **24** and **25**, there was a significant drop in anti-bacterial activity which mirrors what we saw in the acridone and xanthone series. This however was not the case in the 9-octylamino compounds **23** and **28,** which showed no significant drop off between the epoxy and diol analogs which suggests these particular analogs are unique in the way they are killing the bacteria.

We believe this is due to the unique nature of long chain 9-alkylamino groups on acridines. In another study which looked at the effect of differing length of 9-alkylamino substituent's on acridines, we found that the longer the tail length, the higher the anti-bacterial activity up until the alkylamino group reaches between 9 and 12 carbons, depending on the other substitutions on the acridine (see previous chapter). While the normally accepted mechanism of action of the acridines, both against cancer and bacteria is the DNA via its ability to intercalate, we found that the most potent anti-bacterial compounds were also the worst intercalators, which suggests the anti-bacterial activity is driven by the 9-alkylamino group. This appears to be the case as well for compounds **23** and **28** as they did not show a significant drop in activity between the epoxide and diol like the other analogs did. In the case of these analogs, it does not appear that the addition of an epoxide group has any effect on the activity of the compounds as it does in the other acridine, acridone, and xanthone analogs.

Conclusions

We have presented here the anti-bacterial activity and vero cell toxicity of acridine, acridone, and xanthone based compounds bearing either 1,2-epoxypropane or 1,2-propanediol substituents. When examining the acridone and xanthone compounds which are generally not believed to be compounds that intercalate DNA,[103] the addition of two epoxy groups at the 1- and 3- position exhibited good anti-bacterial activity with moderate toxicity. In an attempt to elucidate the mechanism of action of these compounds, they were tested in an antisense RNA sensitization assay against a small library of *t*RNA synthetases along with the A and B subunits of DNA gyrase and bacterial topoisomerase IV. In this set of assays, none of the *t*RNA or enzymes tested for were determined to be the target for the acridone and xanthone epoxides. Given that compound **7** bearing an epoxy group at both the 1- and 3- position showed the highest activity along with a fourfold increase in activity for bacteria over vero cells suggests that while the target for these compounds is unknown, there is something special about the di-epoxy acridone that could make it a useful anti-bacterial agent. More work is warranted to determine the mechanism of action of this particular analog.

In the case of our acridine-based compounds, almost every compound showed some degree of activity. This is likely due to the established target of the DNA and its related enzymes for most acridine based compounds, which makes them less dependent on their substitution to drive their activity. Interestingly in the case of the 4-amino-1-benzyl-piperidine and 1-methyl-tryptamino analogs, the

addition of the epoxide moiety in the 4 position did not showed a marked improvement in anti-bacterial activity or toxicity compared to the un-substituted phenolic acridine, however the ring opening of this epoxide to the 1,2-diol caused a marked loss in activity against the strains of *S. aureus*. Future work to elucidate the mechanism of action of these compounds would be to test them in the anti-sense RNA assay and test the degree of their DNA intercalation to see if they are hitting a different target or if somehow the 1,2-diol group interferes with the ability of the acridine to intercalate into the DNA helix.

In the special case of the 9-octylamino acridines, regardless of the substitution or lack thereof, these compounds maintained excellent activity against *S. aureus* along with an increased toxicity compared to the other acridine compounds. We believe this is due to the special nature of how these long chain 9-alkylamino acridines interact with the cells. Our previous unpublished work suggests that these long chain 9-alkylamino compounds do not intercalate DNA but rather act on some other cellular target. Looking at similar compounds and the literature, we have postulated that these compounds act as cell membrane disruptors due to the cationic acridine "head" and greasy aliphatic tail acting as an amphiphile of sorts in which the tail inserts into the membrane and the positively charged acridine "head" interacts with the high density of negative charges associated with the bacterial cell membrane.[109] We believe this is the case in these compounds as well as our other series due to the fact that unlike the other compounds we have presented in this paper, there is no difference in activity between the phenolic, epoxy, and diol 9-octylamino compounds. Further studies

would be warranted to carry out a cell membrane disruption assay to test this theory and more SAR work needs to be done in an attempt to retain potency while cutting down on the toxicity of these compounds to possibly develop a novel anti-bacterial agent to combat the ever growing threat of drug-resistant bacteria.

Experimental

Biology General

S. aureus strains used in this study include MRSA isolates MW2 (USA400) and 1371 (USA300) and MSSA isolate MSA553. The *S. aureus* cells were cultured in Trypticase soy broth (TSB) at 37˚C with shaking.

S. aureus susceptibility assay

S. aureus strains were grown in TSB at 37°C overnight and were diluted to ~10^5 CFU/ml as cultures for MIC assays with a 96-well microtiter format. Serial dilutions of 40 standard compounds were prepared in TSB-Erm in a final assay volume of 100 μl. Fifty μl of 10^5 CFU/ml bacteria was added to the serially diluted antibiotics. The MIC was considered to be the concentration at which the antibiotic prevented turbidity in the well after incubation for18 h at 37°C. The MIC assay was repeated three times, and the table in the results section represented one experiment.

Cell culture

Vero monkey kidney epithelial cells (ATCC CCL-81) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, CA). Cultures of Vero cells were maintained in a medium containing penicillin (5μg/ml) and streptomycin (100μg/ml) (Invitrogen, CA). Assays were performed in RPMI 1640 medium with different doses of tested compounds.

Cytotoxicity assays

The cytotoxicity assay was conducted by measuring LDH release as described[135]. Briefly, all cells were grown in 96-well plates to 90% confluence. To test the cytotoxicity, monolayer cells were exposed to different doses of tested compounds and incubated for 16 h at 37ºC with 5% CO_2. At the end of the experiment, cell viability was determined by measuring LDH release using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, MI) as per manufacturer's instructions. Each experiment was repeated three times, and all of the percentage of cell death related to control (no death) were calculated.

Chemistry General

1,3-dihydroxyacridin-9(10H)-one (4): A mixture of methyl anthranilate (6.6 mmol), phloroglucinol (6.6 mmol), and p-toluene-sulfonic acid (100 mg) in 40 mL of hexanol was heated at reflux for 4 h. The reaction mixture was then cooled, petroleum ether was added, and the mixture stirred well. The product was then filtered and washed with petroleum ether followed by dichloromethane. The

resultant solid was recrystallized from ethanol/water to yield 1.28 g (5.63 mmol) of a yellow solid. 85% yield. ^{1}H-NMR (DMSO-d_6): 5.97 (d, *J* = 1.2 Hz, 1H), 6.26 (d, *J* = 0.6 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.68 (t, *J* = 7.8, 7.2 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 10.49 (s, 1H), 11.72 (s, 1H), 14.72 (s, 1H).

General procedure for mono and di-epoxy-acridones: 1,3-dihydroxyacridin-9(10H)-one (1.1 mmol) and Cs_2CO_3 (2.2 mmol) was dissolved in 40 mL of a 1:2 DMF:Acetone mixture. Epibromohydrin (1.23 mmol) was added and the reaction was heated at 40 °C for 16 hours. The reaction was cooled and diluted with water, then extracted with ethyl acetate. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified on silica eluting 2:1 Hex:EtOAc to afford both the mono-epoxy and di-epoxy acridone as yellow solids.

1-hydroxy-3-(oxiran-2-ylmethoxy)acridin-9(10H)-one (6)

52% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 2.74 (m, 1H), 2.86 (t, *J* = 4.8 Hz, 1H), 3.35 (m, 1H), 3.92 (m, 1H), 4.42-4.45 (dd, *J* = 2.4, 9.0 Hz, 1H), 6.18 (d, *J* = 2.4 Hz, 1H), 6.37 (s, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 11.9 (s, 1H), 14.22 (s, 1H); HRMS ($C_{16}H_{13}NO_4$) $[M-H]^-$: found *m*/*z* 282.0741, calcd 282.0772

1,3-bis(oxiran-2-ylmethoxy)acridin-9(10H)-one (7)

22% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 2.63 (m, 1H), 2.74 (m, 1H), 2.82 (m, 1H), 2.87 (m, 1H), 3.37 (m, 1H), 3.45 (m, 1H), 3.98 (m, 1H), 4.44-4.52 (m,

2H), 4.97 (dd, 1H) 6.33 (d, *J* = 2.4 Hz 1H), 6.67 (t, *J* = 1.8 Hz, 1H), 7.35 (t, *J* = 6.6 Hz, 1H), 7.82 (m, 2H), 8.28 (d, *J* = 6.6 Hz, 1H); HRMS ($C_{19}H_{17}NO_5$) $[M+H]^+$: found *m*/*z* 340.1184, calcd 340.1179.

1,3-bis(oxiran-2-ylmethoxy)-10-(oxiran-2-ylmethyl)acridin-9(10H)-one (8)

1,3-dihydroxyacridin-9(10H)-one (2.2 mmol) and Cs_2CO_3 (11 mmol) was dissolved in 20 mL of DMF. Epibromohydrin (22 mmol) was added and the reaction was heated at 70 °C for 16 hours. The reaction was cooled and diluted with water, then extracted with ethyl acetate. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified on silica eluting 2:1 Hex:EtOAc to yield a yellow solid. 26% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 2.65 (m, 1H), 2.74 (m, 1H), 2.84-2.89 (m, 3H), 3.08 (m, 1H), 3.39 (m, 2H), 3.46 (m, 1H), 4.02 (m, 2H), 4.37 (m, 2H), 4.51 (d, *J* = 11.4 Hz, 1H), 4.88-4.91 (dd, *J* = 4.8, 12.0 Hz, 1H), 6.45 (s, 1H), 6.76 (s, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.68 (m, 2H), 8.20 (d, *J* = 7.2 Hz, 1H); HRMS ($C_{22}H_{21}NO_6$) $[M+H]^+$: found *m*/*z* 396.1543, calcd 396.1520

3-(2,3-dihydroxypropoxy)-1-hydroxyacridin-9(10H)-one (11): 1-hydroxy-3-(oxiran-2-ylmethoxy)acridin-9(10H)-one (0.25 mmol) was dissolved in 15 mL of 20% aqueous NaOH and stirred at 70 °C for 16 hours. The reaction was then cooled and concentrated. The residue was taken up in EtOAc, washed with water, dried over Na_2SO_4 and concentrated in vacuo followed by purification on silica eluting 95:5 DCM:MeOH to yield the compound as a yellow solid. 78% yield. ^{1}H-NMR (600 MHz, CD_3OD): δ 3.65-3.82 (m, 2H), 3.9-4.16 (m, 3H), 6.18

(s, 1H), 6.36 (s, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 1H); HRMS ($C_{16}H_{15}NO_5$) $[M-H]^-$: found *m*/*z* 300.0864, calcd 300.0877.

1,3-bis(2,3-dihydroxypropoxy)acridin-9(10H)-one (12): 1,3-bis(oxiran-2-ylmethoxy)-10-(oxiran-2-ylmethyl)acridin-9(10H)-one (0.15 mmol) was dissolved in 15 mL of 20% aqueous NaOH and stirred at 70 °C for 16 hours. The reaction was then cooled and concentrated. The residue was taken up in EtOAc, washed with water, dried over Na_2SO_4 and concentrated in vacuo followed by purification on silica eluting 95:5 DCM:MeOH to yield the compound as a yellow solid. 80% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 3.44-3.58 (m, 4H), 3.81 (m, 1H), 4.00 (m, 2H), 4.13 (m, 1H), 4.47 (m, 2H), 6.26 (s, 1H), 6.76 (s, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.79 (t, *J* = 7.2 Hz, 1H), 7.96 (m, 1H), 8.29 (d, *J* = 7.8 Hz, 1H). HRMS ($C_{19}H_{21}NO_7$) $[M-H]^-$: found *m*/*z* 374.1229, calcd 374.1234.

1,3-dihydroxy-9H-xanthen-9-one (5): Salicylic acid (36.2 mmol), phloroglucinol (55.5 mmol), and zinc (110 mmol) were dissolved in 35 mL of phosphorous oxychloride and stirred at 70 °C for 2 hours. After 2 hours, the mixture was cooled and poured into ice water. The resultant precipitate was collected via filtration, washed with water, and dried. The compound was purified on silica eluting 4:1 Hex:EtOAc to yield the compound as a light yellow solid. 42% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.24 (d, *J* = 2.4 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 7.45 (t, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.84 (m, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 11.09 (s, 1H), 12.80 (s, 1H); HRMS ($C_{13}H_8O_4$) $[M-H]^-$: found *m*/*z* 227.0336, calcd 227.0350.

General procedure for mono and di-epoxy-xanthones: 1,3-dihydroxy-9H-xanthen-9-one (0.88 mmol) and Cs_2CO_3 (1.31 mmol) was dissolved in 10 mL of a 1:1 DMF:Acetone mixture. Epibromohydrin (1.23 mmol) was added and the reaction was heated at 60 °C for 16 hours. The reaction was cooled and diluted with water, then extracted with ethyl acetate. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified on silica eluting 2:1 Hex:EtOAc to afford both the mono-epoxy and di-epoxy xanthone as pale yellow solids.

1-hydroxy-3-(oxiran-2-ylmethoxy)-9H-xanthen-9-one (9): 40% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 2.71 (m, 1H), 2.85 (t, *J* = 4.2 Hz, 1H), 3.35 (d, *J* = 2.4 Hz, 1H), 3.96 (m, 1H), 4.50 (d, *J* = 11.4 Hz, 1H), 6.42 (s,, 1H), 6.66 (s, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.86 (t, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H); HRMS ($C_{16}H_{12}O_5$) $[M-H]^-$: found *m*/*z* 283.0632, calcd 283.0612.

1,3-bis(oxiran-2-ylmethoxy)-9H-xanthen-9-one (10): 47% yield. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 2.72 (m, 1H), 2.85 (m, 2H), 3.02 (m, 1H), 3.37 (m, 2H), 3.96 (m, 1H), 4.03 (m, 1H), 4.43 (d, *J* = 11.4 Hz, 1H), 4.50 (d, *J* = 11.4 Hz, 1H) 6.53 (s, 1H), 6.70 (s, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.73 (t, *J* = 7.2 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H). HRMS ($C_{19}H_{16}O_6$) $[M-H]^-$: found *m*/*z* 341.1052, calcd 341.1020.

Methyl-2-iodobenzoate (14): 2-iodobenzoic acid (20.16 mmol) was dissolved in 50mL DMF and 1.2 eq. of potassium carbonate (24.19 mmol) was added. This mixture was stirred at room temperature for 30 min. 1.2 eq of methyl iodide

(24.19 mmol) was then added drop wise and this mixture was stirred at room temp for 1 hour. The reaction mixture was diluted in 250mL of diethyl ether and washed 3x250mL of water. The organic layer concentrated to afford 5.16g of a yellowish oil (19.69 mmol). 98% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H), 7.26 (td, *J* = 7.7, 1.7 Hz, 1H), 7.49 (td, *J* = 7.6, 1.2 Hz, 1H), 7.70 (dd, *J* = 7.8, 1.8 Hz, 1H), 8.00 (dd, *J* = 7.9, 1.1, 1H).

General Procedure for (2-methoxyphenylamino)benzoates: Compounds were prepared by a modified procedure found in the literature.[7] ($Pd(OAc)_2$ (3 mol%), dpff (6 mol%) and *p*-anisidine (45.5 mmol) were dissolved in 100 mL dry toluene and stirred under argon at 95 °C for 15 min. After 15 min, methyl-2-iodobenzoate (38 mmol) and Cs_2CO_3 (53.2 mmol) were added. The reaction mixture was stirred at 95° C for 10 hours. After cooling, the reaction was filtered and concentrated in vacuo. The resultant oil was adsorbed on celite and purified on silica eluting 10% EtOAc:Hexanes to 20% EtOAc:Hexanes to afford the compounds as yellow oils.

Methyl-2-(4-methoxyphenylamino)benzoate: 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (s, 3H), 3.90 (s, 3H), 6.66 (t, *J* = 7.3 Hz, 1H), 6.88-6.93 (m, 2H), 6.97 (d, *J* = 8.6 Hz, 1H), 7.15-7.19 (m, 2H), 7.25 (m, 1H), 7.94 (dd, *J* = 8.0, 1.6 Hz, 1H), 9.26 (br, s, 1H).

Methyl-2-(2-methoxyphenylamino)benzoate: Yield not determined and compound not characterized due to flask breaking in the hood. The yellow oil was recovered from the bottom of the hood and carried on without re-purification.

2-(2-methoxyphenylamino)benzoic acid (15a): An unknown amount of methyl-2-(2-methoxyphenylamino)benzoate was dissolved in 500 mL of acetone. 350 mL of 5% NaOH was added and the reaction was stirred at room temperature overnight. The reaction was then neutralized with 3N HCl and the organics were removed under reduced pressure. The resultant yellow solid was collected via filtration and washed 2x with 150mL water to yield 7.50 g of orangeish-yellow solid. Yield not determined. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.76 (t, *J* = 7.4 Hz, 1H), 6.94 (m, 1H), 7.01-7.11 (m, 2H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.34-7.41 (m, 2H), 7.89 (dd, *J* = 7.9, 1.5 Hz, 1H), 9.58 (s, 1H), 12.97 (br, s, 1H).

2-(4-methoxyphenylamino)benzoic acid (15b):

Methyl-2-(4-methoxyphenylamino)benzoate (37.53 mmol) was dissolved in 500 mL of acetone. 350 mL of 5% NaOH was added and the reaction was stirred at room temperature overnight. The reaction was then neutralized with 3N HCl and the organics were removed under reduced pressure. The resultant yellow solid was collected via filtration and washed 2x with 150mL water to yield 8.02g (32.97 mmol) of yellow solid. 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (s, 3H), 6.68 (t, *J* = 7.6 Hz, 1H), 6.89-6.99 (m, 3H), 7.15-7.21 (m, 2H), 7.32 (ddd, *J* = 8.6, 7.0, 1.7 Hz, 1H), 7.86 (dd, *J* = 7.9, 1.7 Hz, 1H), 9.43 (br, s, 1H), 12.94 (br, s, 1H).

2-methoxy-9-phenoxyacridine (19a): Compound was synthesized using previously published procedures.[103] 76% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.81 (s, 3H), 6.87 (d, *J* = 8.8 Hz, 2H), 7.05 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 2.9 Hz,

1H), 7.25-7.29 (m, 2H), 7.41-7.48 (m, 2H), 7.71 (m, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 8.15 (d, *J* = 9.4 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H).

4-methoxy-9-phenoxyacridine (19b): Compound was synthesized using previously published procedures.[103] 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (s, 3H), 6.83 (dd, *J* = 8.7, 0.9 Hz, 2H), 7.00-7.08 (m, 2H), 7.22-7.30 (m, 2H), 7.36 (dd, *J* = 8.7, 7.5 Hz, 1H), 7.46 (ddd, *J* = 8.6, 6.7, 1.0 Hz, 1H), 7.66 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.76 (ddd, *J* = 8.6, 6.8, 1.4 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 8.43 (d, *J* = 8.8 Hz, 1H).

2-hydroxy-9-phenoxyacridine (20): 3.1 g (10 mmol) of 2-methoxy-9-phenoxyacridine was dissolved in DCM and placed under nitrogen. 4.75 mL (50 mmol) of boron tribromide was added via syringe and the reaction mixture was stirred at room temperature overnight. The reaction was then cooled in an ice bath and carefully quenched with saturated sodium bicarbonate until the evolution of gas ceased and the aqueous phase was at pH 7.0. The aqueous phase was extracted with DCM and the combined organics were concentrated and purified on silica eluting 10% MeOH:DCM to afford the product as a light yellow solid. 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82-6.89 (m, 2H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.28-7.36 (m, 2H), 7.45-7.57 (m, 2H), 7.76 (m, 1H), 7.94 (dd, *J* = 8.7, 0.6 Hz, 1H), 8.12 (d, *J* = 9.4 Hz, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 10.33 (br, s, 1H); HRMS ($C_{19}H_{14}NO_2$) $[M+H]^+$: found *m/z* 288.1021, calcd 288.1019.

4-hydroxy-9-phenoxyacridine (21): Compound was synthesized following the same procedure as compound **20**. 68% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.83-6.90 (m, 2H), 7.06 (m, 1H), 7.14 (dd, *J* = 6.2, 2.2 Hz, 1H), 7.28-7.34 (m, 2H), 7.37-7.45 (m, 2H), 7.59 (ddd, *J* = 8.7, 6.7, 1.1 Hz, 1H), 7.87 (m, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 10.03 (s, 1H); HRMS ($C_{19}H_{14}NO_2$) $[M+H]^+$: found *m/z* 288.1028, calcd 288.1019.

2-(1-methyl-1*H*-indol-3-yl)-ethanamine: Compound was prepared and purified according to literature procedures[131].

General Procedure for 9-alkylamino-hydroxy acridines: 280 mg (1 mmol) of 4-hydroxy-9-phenoxyacridine and 156 mg (1.2 mmol) of octylamine were dissolved in excess phenol and stirred at 100 °C for 1 hour. Upon cooling, the reaction was diluted in EtOAc and purified on silica eluting 25% MeOH:EtOAc to afford the product as a reddish orange solid.

9-(octylamino)acridin-2-ol (22a): ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 0.84 (t, *J* = 6.5 Hz, 3H), 1.16-1.34 (m, 8H), 1.41 (quin, *J* = 7.1 Hz, 2H), 1.80 (quin, *J* = 7.4 Hz, 2H), 3.80 (t, *J* = 7.2 Hz, 2H), 7.36 (m, 1H), 7.44 (dd, *J* = 9.4, 2.0 Hz, 1H), 7.62 (m, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.96 (m, 2H), 8.33 (d, *J* = 9.0 Hz, 1H); HRMS ($C_{21}H_{27}N_2O$) $[M+H]^+$: found *m/z* 323.2117, calcd 323.2118.

9-(2-(1-methyl-1*H*-indol-3-yl)ethylamino)acridin-2-ol (22b): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.27 (t, *J* = 7.4 Hz, 2H), 3.69 (s, 3H), 4.27 (t, *J* = 7.0 Hz, 2H), 6.96 (t, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.19 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.52-7.62 (m, 2H), 7.79 -7.92 (m, 4H), 8.49 (d, *J* =

8.6 Hz, 1H), 10.30 (br, s, 1H); HRMS ($C_{24}H_{23}N_3O$) $[M+H]^+$: found *m/z* 368.1747, calcd 368.1757.

9-(1-benzylipiperidin-4-ylamino)-acridin-2-ol (22c): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.78-2.00 (m, 6H), 2.80 (d, *J* = 10.8 Hz, 2H), 3.43 (s, 2H), 3.65 (br, s, 1H), 6.21 (br, s, 1H), 7.18-7.32 (m, 5H), 7.37 (m, 2H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.84 (t, *J* = 10.0 Hz, 2H), 8.30 (d, *J* = 8.6 Hz, 1H), 9.90 (br, s, 1H); HRMS ($C_{25}H_{26}N_3O$) $[M+H]^+$: found *m/z* 384.2063, calcd 384.2070.

9-(octylamino)acridin-4-ol (22d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.20-1.39 (m, 8H), 1.45 (quin, *J* = 7.3 Hz, 2H), 1.79 (m, 2H), 3.86 (m, 2H), 5.16 (s, 1H), 5.90 (br, s, 1H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.26 (m, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.66 (m, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 8.10 (d, *J* = 8.6 Hz, 1H); HRMS ($C_{21}H_{27}N_2O$) $[M+H]^+$: found *m/z* 323.2119, calcd 323.2118.

9-(2-(1-methyl-1*H*-indol-3-yl)ethylamino)acridin-4-ol (22e): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (t, *J* = 6.6 Hz, 2H), 3.77 (s, 3H), 4.25 (t, *J* = 6.6 Hz, 2H), 5.56 (br, s, 1H), 6.93 (s, 1H), 7.10 (d, *J* = 7.4 Hz, 1H), 7.15-.722 (m, 2H), 7.30 (m, 2H), 7.37 (m, 2H), 7.65 (m, 2H), 7.96 (d, *J* = 8.8 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{24}H_{23}N_3O$) $[M+H]^+$: found *m/z* 368.1754, calcd 368.1757.

9-(1-benzylipiperidin-4-ylamino)-acridin-4-ol (22f): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69-1.80 (m, 2H), 2.04-2.16 (m, 4H), 2.85-2.91 (m, 2H), 3.52 (s, 2H), 3.94 (br, s, 1H), 4.79 (br, s, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.26 (m, 1H), 7.28-7.35 (m, 5H),

7.43 (t, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.69 (m, 1H), 8.08 (t, *J* = 7.5 Hz, 2H); HRMS ($C_{25}H_{26}N_3O$) $[M+H]^+$: found *m/z* 384.2066, calcd 384.2070.

General Procedure for 2-epoxy acridines: 325 mg (1 mmol) of 9-(octylamino)acridin-2-ol was dissolved in 40 mL 1:1 DMF:MeCN and 650 mg (2 mmol) Cs_2CO_3 was added. The mixture was stirred at 70 °C for 10 min. 165 mg (1.2 mmol) of epibromohydrin was added and the reaction was stirred at 70 °C for 12 hours. Upon cooling, the reaction was passed through a plug of celite and solvent was removed by rotary evaporation. The residue was purified on silica eluting 20:80 MeOH:EtOAc to 20:75:5 MeOH:EtOAc:Et_3N. Collected fractions were concentrated, taken up into DCM and precipitated with hexanes to afford the compound as a yellow solid.

***N*-octyl-2-(oxiran-2-ylmethoxy)-acridin-9-amine (23)** ^1H NMR (400 MHz, $CDCl_3$) δ 0,89 (m, 3H), 1.24-1.48 (m, 8H), 1.56 (m, 2H), 2.07 (m, 2H), 2.90 (dd, *J* = 4.8, 2.6 Hz, 1H), 2.97 (m, 1H), 3.40 (m, 1H), 4.04-4.15 (m, 3H), 4.63 (d, *J* = 11.7 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 7.28 (m, 1H), 7.56 (m, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 9.4 Hz, 1H), 8.07 (dd, *J* = 11.7, 9.0 Hz, 2H); HRMS ($C_{24}H_{31}N_2O_2$) $[M+H]^+$: found *m/z* 379.2385, calcd 379.2380.

N-(2-(1-methyl-1H-indol-3-yl)ethyl)-2-(oxiran-2-ylmethoxy)acridin-9-amine (24) ^1H NMR (400 MHz, $(CD_3)_2CO$) δ 2.72 (dd, *J* = 5.1, 2.5 Hz, 1H), 2.86 (dd, *J* = 5.1, 4.3 Hz, 1H), 3.22 (t, *J* = 7.1 Hz, 2H), 3.35 (qd, *J* = 4.4, 2.7 Hz, 1H), 3.73 (s, 3H), 3.88 (dd, *J* = 11.2, 6.3 Hz, 1H), 4.14 (t, *J* = 7.1 Hz, 2H), 4.28 (dd, *J* = 11.1, 2.8 Hz, 1H), 7.01 (m, 1H), 7.06 (s, 1H), 7.15 (m, 2H), 7.36 (m, 2H), 7.41 (dd, *J* =

9.4, 2.7 Hz, 1H), 7.51 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.63 (ddd, *J* = 8.5, 6.8, 1.4 Hz, 1H), 7.96 (m, 2H), 8.29 (d, *J* = 8.2 Hz, 1H); HRMS ($C_{27}H_{26}N_3O_2$) $[M+H]^+$: found *m/z* 424.2017, calcd 424.2020.

N-(1-benzylpiperidin-4-yl)-2-(oxiran-2-ylmethoxy)acridin-9-amine (25) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70-1.79 (m, 2H), 1.99-2.09 (m, 4H), 2.83-2.92 (m, 3H), 2.99 (t, *J* = 4.4 Hz, 1H), 3.50 (s, 2H), 3.72 (br, s, 1H), 4.09 (dd, *J* = 10.6, 5.9 Hz, 1H), 4.36 (d, *J* = 10.6 Hz, 1H), 4.41 (d, *J* = 11.2 Hz, 1H), 7.26 (m, 1H), 7.28-7.34 (m, 5H), 7.41-7.48 (m, 2H), 7.66 (t, *J* = 7.3 Hz, 1H), 8.03-8.08 (m, 2H), 8.11 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{28}H_{30}N_3O_2$) $[M+H]^+$: found *m/z* 440.2337, calcd 440.2333.

3-((9-phenoxyacridin-2-yl)oxy)propane-1,2-diol (26): 280 mg (1 mmol) of 2-hydroxy-9-phenoxyacridine was dissolved in 40 mL 1:1 DMF:MeCN and 650 mg (2 mmol) Cs_2CO_3 was added. The mixture was stirred at 70 °C for 10 min. 133 mg (1.2 mmol) of 3-chloro-1,2-propanediol was added and the reaction was stirred at 70 °C for 12 hours. Upon cooling, the reaction was passed through a plug of celite and solvent was removed by rotary evaporation. The residue was passed through a silica plug eluting 20:80 MeOH:EtOAc and carried on without further purification.

3-((9-phenoxyacridin-4-yl)oxy)propane-1,2-diol (27): Followed the same procedure for 3-((9-phenoxyacridin-2-yl)oxy)propane-1,2-diol.

3-(9-(octylamino)acridin-2-yl)oxy)propane-1,2-diol (28) ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.19-1.35 (m, 8H), 1.40 (m, 2H), 1.73 (quin, *J* =

7.3 Hz, 2H), 3.66 (t, *J* = 7.2 Hz, 2H), 3.86 (m, 1H), 3.93 (m, 1H), 4.15-4.25 (m, 3H), 4.79 (br, s, 2H), 7.21 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 9.5, 2.4 Hz, 1H), 7.40 (m, 1H), 7.65 (m, 1H), 7.97 (d, *J* = 9.4 Hz, 1H), 8.07 (t, *J* = 9.8 Hz, 2H); HRMS ($C_{24}H_{33}N_2O_3$) $[M+H]^+$: found *m/z* 397.2491, calcd 397.2486.

3-(9-(2-(1-methyl-1H-indol-3-yl)ethylamino)acridin-2-yl)oxy)propane-1,2-diol (29) ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.11 (t, *J* = 7.6 Hz, 2H), 3.53 (t, *J* = 5.1 Hz, 2H), 3.68 (s, 3H), 3.90 (m, 1H), 3.97-4.07 (m, 3H), 4.15 (dd, *J* = 9.9, 4.2 Hz, 1H), 4.75 (t, *J* = 5.5 Hz, 1H), 5.05 (d, *J* = 5.1 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 7.08-7.14 (m, 2H), 7.29-7.37 (m, 2H), 7.41 (d, *J* = 9.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.58-7.66 (m, 2H), 7.78-7.90 (m, 2H), 8.30 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{27}H_{28}N_3O_3$) $[M+H]^+$: found *m/z* 442.2107, calcd 442.2125.

3-(9-(1-benzylpiperidin-4-ylamino)acridin-2-yl)oxy)propane-1,2-diol (30) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70-1.82 (m, 2H), 1.95-2.10 (m, 4H), 2.85-2.93 (m, 2H), 3.51 (s, 2H), 3.71 (br, s, 1H), 3.84-3.98 (m, 2H), 4.16-4.27 (m, 3H), 4.57 (br, s, 2H), 7.21 (d, *J* = 2.5 Hz, 1H), 7.28-7.36 (m, 5H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.99 (d, *J* = 9.4 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 8.11 (d, *J* = 8.6 Hz, 1H); HRMS ($C_{28}H_{32}N_3O_3$) $[M+H]^+$: found *m/z* 458.2442, calcd 458.2438.

3-(9-(octylamino)acridin-4-yl)oxy)propane-1,2-diol (31) ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.21-1.40 (m, 8H), 1.46 (m, 2H), 1.81 (m, 2H), 1.81 (quin, *J* = 7.1 Hz, 2H), 3.87 (t, *J* = 7.4 Hz, 2H), 3.93 (m, 2H), 4.14 (m, 1H), 4.27 (dd, *J* = 10.0, 5.7, 1H), 4.45 (dd, *J* = 10.0, 2.5 Hz, 1H), 5.37 (br, s, 2H), 7.20 (m, 1H), 7.29 (m, 1H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.66 (m, 1H), 7.78 (d, *J* = 8.6 Hz,

1H), 8.08 (d, *J* = 8.8 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 1H); HRMS ($C_{24}H_{33}N_2O_3$) $[M+H]^+$: found *m/z* 397.2505, calcd 397.2486.

3-(9-(2-(1-methyl-1H-indol-3-yl)ethylamino)acridin-4-yl)oxy)propane-1,2-diol (32) ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.28 (t, *J* = 7.1 Hz, 2H), 3.68-3.82 (m, 5H), 4.04 (m, 1H), 4.20-4.29 (m, 3H), 4.31 (m, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 7.10 (s, 1H), 7.14 (m, 1H), 7.18-7.36 (m, 4H), 7.58-7.68 (m, 2H), 7.92-8.01 (m, 2H), 8.29 (d, *J* = 8.6 Hz, 1H); HRMS ($C_{27}H_{28}N_3O_3$) $[M+H]^+$: found *m/z* 442.2146, calcd 442.2125.

3-(9-(1-benzylpiperidin-4-ylamino)acridin-4-yl)oxy)propane-1,2-diol (33) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76 (m, 2H), 2.01-2.15 (m, 4H), 2.88 (d, *J* = 11.8 Hz, 2H), 3.52 (s, 2H), 3.84-3.98 (m, 3H), 4.13 (m, 1H), 4.29 (dd, *J* = 10.0, 5.5 Hz, 1H), 4.48 (dd, *J* = 9.9, 2.4 Hz, 1H), 4.79 (br, s, 2H), 7.26 (m, 2H), 7.29-7.36 (m, 5H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.66-7.76 (m, 2H), 8.05 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.8 Hz, 1H); HRMS ($C_{28}H_{32}N_3O_3$) $[M+H]^+$: found *m/z* 458.2442, calcd 458.2440.

Chapter 5: Synthesis of Bis-Acridines and Cytotoxic Evaluation in the DU145 Prostate Cancer Cell Line

Introduction

DNA is a well known and well studied target for the development of potential novel anti-cancer therapies. There are numerous ways in which drugs can interact with DNA and exert their anti-cancer effects. Cross-linking agents such as Mitomycin C[136] and Cisplatin[137] alkylate two complimentary DNA strands, prevent DNA replication, and lead to cell death. DNA groove binders such as Distamycin A exert their cytotoxic effects by non-covalently binding the minor groove and preventing gene transcription or DNA replication.[138] There are drugs that cause DNA damage via cleaving the DNA strand, drugs that incorporate into the replicating DNA that block chain elongation, and drugs that intercalate DNA, interfering with the enzymes responsible for modifying the topology of DNA and replicating it.[139] It is the last class, the intercalators, that has received the most attention over the years and is the class of compounds that the acridines belong to.

Acridines are tri-cyclic, planar heterocycles that reversibly bind DNA via intercalation.[140] The 9-amino acridines are cationic at physiological pH which enhances their binding to the DNA backbone via ionic interaction between the positively charged nitrogen of the acridine and the negatively charged phosphate backbone of the DNA.[141] The acridines previously described by our laboratory act via DNA intercalation that interferes with the catalytic cycle of topoisomerase

II.[115] Despite their effectiveness, acridines still have numerous downsides such as high toxicity due to non-specific intercalation[142] and short lived complexes with the DNA.[143] One strategy to enhance the binding of acridines to DNA and form longer lived complexes with potentially a higher degree of selectivity is to link two acridines into a complex capable of bis-intercalation.[144,145]

The first bis-intercalator discovered, echinomycin, is a symmetrical peptide anti-biotic with two quinoxaline chromophores. Evidence that suggested echinomycin was a bis-intercalator came from research comparing the perturbation of the DNA helix compared to a known mono-intercalator and it was found to cause the helix to extend twice of what would be expected for a mono-intercalating agent.[146] This discovery has spurred numerous studies into trying to enhance the potency and selectivity of intercalative drugs by linking them in a manner that would allow for bis-intercalation.[147]

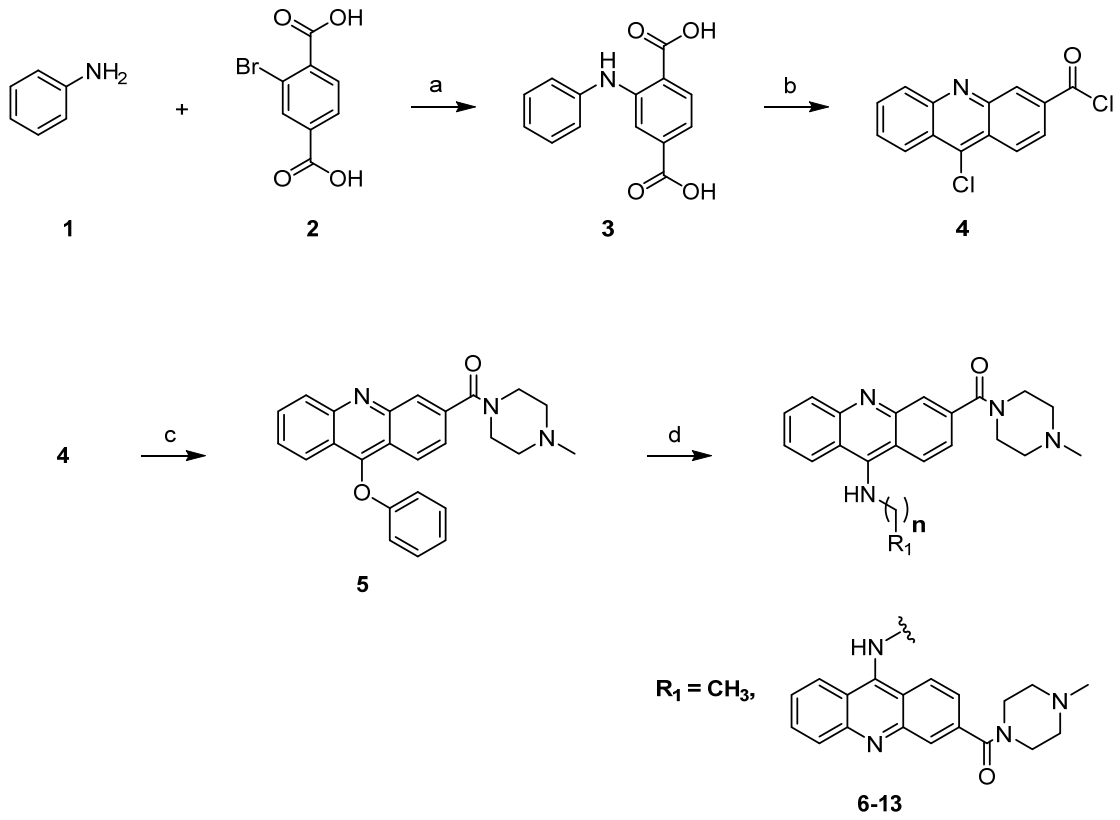
Following the principles on bis-intercalation found in these numerous studies, we set out to link together our most promising acridine chromophores in a manner that would hopefully lead to them becoming bis-intercalating anti-cancer agents. To do this, we chose a simple symmetrical di-amine starting with a linker length of six atoms. This length was chosen as it has been reported that it is the minimum linker length that would facilitate bis-intercalation without violating the neighbor exclusion principal, which states two intercalators cannot intercalate between adjacent base pairs.[147] Along with the bis-acridines, we needed a set of control analogs that lack the second chromophores yet retain a similar linker. To

accomplish this, we decided the best control molecules would be the acridine core with the appropriate length alkylamine at the 9-position.

Synthesis

Shown in **Scheme 5**, synthesis of the bis-acridines and their controls started with construction of the acridine core itself. To accomplish this, I followed work previously pioneered in our lab by Dr. John Goodell that utilized an Ullmann-Jourdan condensation between 2-bromoterephthalic acid **1** and aniline **2** to afford 2-phenylaminoterephthalic acid **3.** Tandem cyclization and acyl chloride formation in refluxing phosphorus oxychloride gave us intermediate **4** which was immediately reacted with *N*-methylpiperizine yielding a 9-chloro-3-carboxamide acridine that was converted to its more stable 9-phenoxy-3-carboxamide acridine **5** by reaction with molten phenol. With our intermediate in hand for the synthesis of all the analogs, our first attempts to synthesize the bis-acridines were unsuccessful. Ideally we would have been able to continue following the methodology previously developed in our lab, however due to the nature of the bis-acridine, precise control over the equivalents of acridine and amine are vital to the success of the synthesis. This meant that running the reaction neat was not a viable route due to the small amount of di-amine needed. This lead us to try a number of solvents ranging from dichloromethane to xylenes across the range of their boiling points. None of these reaction conditions afforded our desired bis-acridine products due to no reaction occurring when followed by

mass spec. Eventually it was found that using phenol as the solvent with a ratio of 2.1 equivalents of acridine to 1 equivalent of di-amine gave low to moderate yields of the bis-acridines **6-9**. The corresponding control acridines **10-13** were synthesized as described in chapter 2.



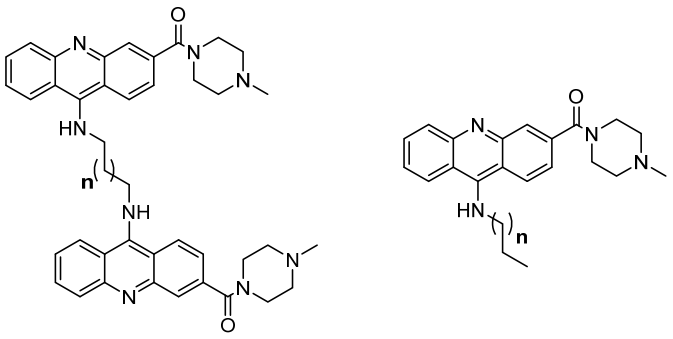
Scheme 5: Synthesis of Bis-Acridines and controls: Reagents and conditions: (a) Cu, CuI, Pyridine, K_2CO_3, H_2O, reflux, 4h; (b) $POCl_3$, reflux, 2h; (c) i. *N*-methylpiperizine, DCM, 0 °C to rt, 1h, ii. Phenol, 80 °C, 2h; (d) amine, phenol, 100 °C, 1-4h.

Biological Evaluation

With the desired bis-acridines and controls in hand, we tested the compounds for their cytotoxic activity against the DU145 prostate cancer cell line using a Cell Titer-Blue® assay.[148]

Results and Discussion

Shown in **Table 4** are the cytotoxicity results for the 8 compounds synthesized and tested in this study. This bis-acridines **6-9** showed increasing activity with the increase in linker length between the acridine chromophores. This result was as expected, as we hypothesized the longer the linker, the more likely the compound would be to bis-intercalate and be more potent. When comparing the cytotoxicity to the control acridines **10-13**, the compounds showed the same trend in increasing cytotoxicity with substituent length, however there was not a marked loss in activity when compared to the respective bis-acridine compound. This suggests to us that linking our 3-carboxamide acridines confers no advantages over using a simple alkylamine in the 9 position.



6-9
n=4-7

10-13
n-4-7

DU145 Prostate Cancer Cell Cytotoxicity

Compound	n =	EC_{50} (μM)	Compound	n =	EC_{50} (μM)
6	4	> 100	**10**	4	28.48 ± 2.62
7	5	50.20 ± 0.67	**11**	5	19.13 ± 6.76
8	6	17.01 ± 3.98	**12**	6	14.22 ± 3.68
9	7	9.22 ± 1.67	**13**	7	6.42 ± 1.29

Table 4: Cytotoxicity results for the bis and control acridines

One possible explanation to this is the position of our carboxamide group. In previously published studies, the 1,8-diaminooctane linker gave the biggest increase in potency over the monomeric acridine, however in all of these studies, the acridine had a 4-carboxamide group instead of the 3-carboxamide group we have.[143,144] One caveat of this is their monomeric controls were just the un-substituted 9-amino-4-carboxamide acridine. When we compare our data in which our controls were much more similar to the bis-compounds than in previously published studies, the nature of the 9-amino substituent may be more important and over-ride any effects gained from having a bis-intercalator. Further work on our compounds determining the DNA binding constants would provide more insight into the importance of having the bis-acridine instead of the simple

9-alkylamino acridine. Perhaps the bis-compounds do bind DNA more tightly with a longer lasting complex in a manner that does not increase their potency, however decreases their general toxicity due to non-specific binding.

Experimental

1. Cytotoxicity Assay

DU145 cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (pen/strep). After reaching confluency, cells were treated with trypsin, pelletized, and re-suspended in growth media. Cell concentration was determined via counting on a hemocytometer and then cells were plated at a concentration of 5000 cells per well and left to incubate for 24 hours. After 24 hours, growth media was removed and the cells were treated with fresh growth media containing 2% DMSO and the compounds starting at 100 μM with a 2 fold dilution factor over 8 wells in triplicate. The treated cells were incubated for 48 hours then viability was tested using the Cell Titer-Blue® assay. Dose response curves and EC_{50} values were calculated using GraphPad Prism 4.0. The assays were repeated on three consecutive days.

2. Synthesis

Compounds **3**, **4**, and **5** were prepared according to previously published procedures.[103]

General Procedure for Synthesis of Bis-Acridines: (4-methylpiperazin-1-yl)(9-phenoxyacridin-3-yl)methanone (2.2 mmol) was dissolved in 10g of molten phenol. 1,6-diaminohexane (1.0 mmol) was added and the reaction was stirred at 100 °C for 4 hours. The reaction was then cooled, dissolved in dichloromethane, and purified on silica eluting 10% MeOH:DCM to 10% MeOH:DCM with 1% triethylamine. The purified product was dissolved in ethyl acetate and precipitated with hexanes to afford the desired compounds as orange solids.

((nonane-1,9-diylbis(azanediyl))bis(acridine-9,3-diyl))bis((4-methylpiperazin-1-yl)methanone) (6). Yield: 31%; ^{1}H NMR (600 MHz, CD_3OD) δ 1.19 (m, 6H), 1.31 (m, 4H), 1.79 (quin ,*J* = 7.3 Hz, 4H), 2.34 (s, 6H), 2.44 (br, s, 4H), 2.56 (br, s, 4H), 3.53 (br, s, 4H), 3.83 (br, s, 4H), 3.93 (t, *J* = 7.2 Hz, 4H), 7.37 (dd, *J* = 1.6, 9.0 Hz, 2H), 7.41 (m, 2H), 7.75 (ddd, *J* = 1.3, 6.9, 8.5 Hz, 2H), 7.83 (m 4H), 8.34 (d, *J* = 8.8 Hz, 2H), 8.44 (d, *J* = 9.1 Hz, 2H).

((octane-1,8-diylbis(azanediyl))bis(acridine-9,3-diyl))bis((4-methylpiperazin-1-yl)methanone) (7). Yield: 26%; ^{1}H NMR (600 MHz, CD_3OD) δ 1.21 (m, 4H), 1.28 (m, 4H), 1.74 (quin, *J* = 7.0, 4H), 2.32 (s, 6H), 2.43 (br, s, 4H), 2.56 (br, s, 4H), 3.53 (br, s, 4H), 3.83 (br, s, 4H), 3.87 (t, *J* = 7.0, 4H), 7.32-7.39 (m, 4H), 7.71 (t, *J* = 7.6 Hz, 2H), 7.81-7.86 (m, 4H), 8.30 (d, *J* = 8.8 Hz, 2H), 8.39 (d, *J* = 8.8 Hz, 2H).

((heptane-1,7-diylbis(azanediyl))bis(acridine-9,3-diyl))bis((4-methylpiperazin-1-yl)methanone) (8). Yield: 38%; ^{1}H NMR (600 MHz,

CD_3OD) δ 1.22-1.32 (m, 6H), 1.70 (m, 4H), 2.31 (s, 6H), 2.41 (br, s, 4H), 2.55 (br, s, 4H), 3.53 (br, s, 4H), 3.75-3.88 (m, 8H), 7.28-7.35 (m, 4H), 7.66 (t, *J* = 7.6 Hz, 2H), 7.82-7.87 (m, 4H), 8.25 (d, *J* = 8.8 Hz, 2H), 8.35 (d, *J* = 8.8 Hz, 2H).

((hexane-1,6-diylbis(azanediyl))bis(acridine-9,3-diyl))bis((4-methylpiperazin-1-yl)methanone) (9). Yield: 34%; ^{1}H NMR (600 MHz, CD_3OD) δ 1.40 (m, 4H), 1.77 (t, *J* = 6.7 Hz, 4H), 2.32 (s, 6H), 2.42 (br, s, 4H), 2.57 (br, s, 4H), 3.53 (br, s, 4H), 3.77-3.90 (m, 8H), 7.28-7.34 (m, 4H), 7.67 (m, 2H), 7.83-7.87 (m, 4H), 8.25 (d, *J* = 8.5 Hz, 2H), 8.36 (d, *J* = 8.8 Hz, 2H).

Chapter 6: Acridine Epoxides; Failures, Lessons, and What Led to the Change in Substitution Pattern

After we had synthesized and tested the acridone and xanthone epoxides and found them to be active against MRSA, we set out to synthesize the analogous acridine epoxides following the same substitution pattern seen in **Figure 16**.

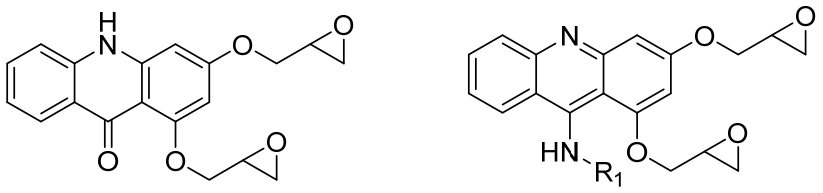


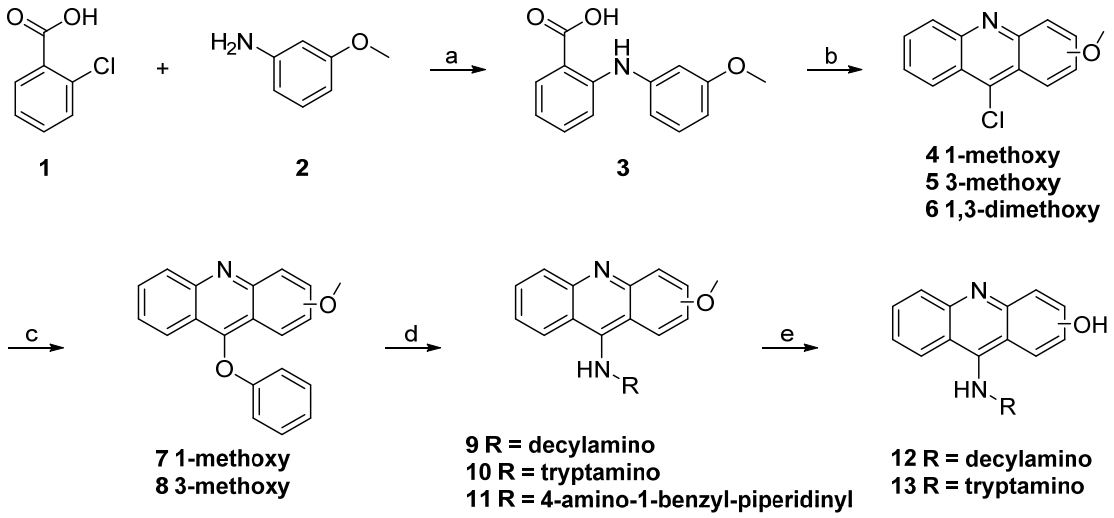
Figure 16: Example acridone epoxide (left) and acridine epoxide (right).

The original synthetic plan for the acridine epoxides called for placing the epoxide groups off hydroxyls located in the 1-, 3-, or both positions of the acridines. We envisioned the most direct route to the desired analogs was via synthesis of the appropriate hydroxy acridines followed by alkylation using epibromohydrin or epichlorohydrin.

Shown in **Scheme 6**, the synthesis started with an Ullmann coupling between *m*-anisidine **1** and 2-chlorobenzoic acid **2** to afford the coupling product **3** which was then cyclize in refluxing phosphorous oxychloride to afford a mixture of 9-chloro-1-methoxy acridine **4** and 9-chloro-3-methoxy acridine **5** which were converted to their more stable 9-phenoxy acridine derivatives **6-7** by heating in molten phenol at 80 °C for 2 hours. The two isomers produced were separable by flash

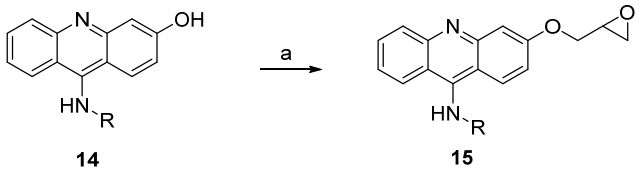
chromatography on silica and gave roughly a 9:1 mix of the 3-substituted product to the 1-substiuted product. Due to the yield of the 1-substituted product being so low, it was set aside and it was decided that most of our work would concentrate on the 3-methoxy acridine.

Reaction of the 9-phenoxy-3-methoxy acridine with either 1-decylamine, tryptamine, or 4-amino-1-benzyl piperidine afforded our 9-amino analogs **9-11**. Demethylation with boron tribromide was successful in the case of the 1-decylamine and tryptamine analogs **9** and **10**, however was unsuccessful in the case of the 4-amino-1-benzyl piperidine analog **11**. Demethylation attempts of compound **11** all led to complex mixtures of compounds, none of which were our desired product.



Scheme 6: Synthesis of 1 and 3 substituted hydroxy-acridines. Reagents and conditions: (a) Cu, CuI, Py, K_2CO_3, H_2O, 110 °C, 4h; (b) $POCl_3$, reflux, 2h; (c) Phenol, 80 °C, 2h; (d) Amine, Phenol, 100 °C, 1h; (e) BBr_3, DCM, rt, 16h.

With two of our desired 9-amino-3-hydroxy acridines in hand, we envisioned a simple alkylation with epibromohydrin (shown in **Scheme 7**) would complete the synthesis of our desired analogs **14** and **15**.



14 **15**

Scheme 7: Synthesis of 3-epoxy acridine analogs. Reagents and conditions: (a) Epibromohydrin, Cs_2CO_3, DMF/Acetone, 70 °C, 16h

What we envisioned as a simple, straightforward reaction turned out to be a very complex issue that was difficult to decipher. When using the same methodology our lab used to make the acridone analogs,[149,150] reaction in a DMF/acetone mixture using cesium carbonate as a base yielded a range of results from inseparable messes to what appeared to be clean compound by mass spectrometry but was a mixture of compounds when the NMR was taken.

Our first thought was that while cesium carbonate in DMF/acetone worked well for acridones and xanthones, that it was not the appropriate base for the acridine system. We proceeded to screen a small number of bases in the alkylation reaction of the various 9-amino-3-hydroxy acridine compounds. Shown in **Table 5** is the result of the base screen. In the small sampling of bases we used, every case in which a non-carbonate base was used, there was no reaction leading to a desired product. When using a carbonate base, switching the solvent to methanol gave no reaction. The only system in which a reaction occurred was the combination of a carbonate base in DMF alone or a DMF/acetone mixture.

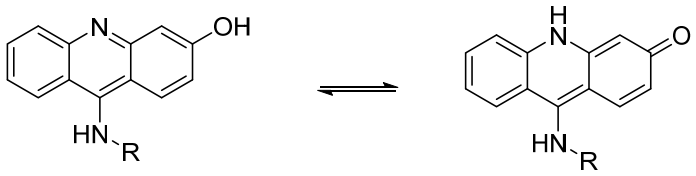
Having ruled out our choice of base as the most probable reason why the reaction was not proceeding as expected, an observation was made about the starting 3-hydroxy acridine compounds that led us to the answer as to why these reactions were not working as we had hoped.

Base	Solvent	Temperature	Time	Result
Cs_2CO_3	DMF	70 °C	16h	Mixture
Cs_2CO_3	DMF/Acetone	50 °C	16h	Mixture
Cs_2CO_3	MeOH	25 °C	6h	No Reaction
K_2CO_3	DMF	70 °C	16h	Mixture
t-BuOK	DMF	50 °C	6h	No Reaction
NaH	DMF	25 °C	6h	No Reaction
Proton Sponge	MeCN	25 °C	6h	No Reaction

Table 5: Base screen results (all reactions were conducted in DMF at 70 °C)

We noticed that depending on how the 3-hydroxy acridine derivatives were handled after purification and what solvent they were dissolved in, they yielded different colored solids or solutions. Sometimes after the 3-hydroxy acridines were purified and precipitated, the precipitate was a yellowish solid and other times it was an orange-red solid. In solution, the colors ranged from a yellowish solution, which showed no ultraviolet hue when held up to natural light to a reddish solution that exhibited a distinct ultraviolet hue similar to that of an acridone in natural light. A closer literature search revealed a reference that had been missed that describes the solid and solution phase tautomerisim of 3-hydroxyacridines[151]. Shown in **Figure 17** are the two forms of the 3-hydroxy acridine; the desired hydroxy form **16** and the keto form **17**. This meant that every time we attempted the alkylation reaction, the 3-hydroxy acridine in the

flask was in a tautomeric equilibrium between the hydroxy and keto form, which led to the complex mixtures of products we were seeing. This is because alkylation was likely occurring on the oxygen in the hydroxy form leading to the desired product and on the nitrogen in the keto form leading to an undesired product. The two products formed were not separable in our hands using either silica gel or alumina flash chromatography. After deciding there was no good way to control which tautomer would be reacting with the alkylating agent, the decision was made to change the substitution to the 2 and 4 hydroxy acridines which do not have possible tautomers.[98]



16 **17**

Figure 17: Tautomerisim of 3-hydroxy acridines

Experimental

All compounds were synthesized using previously published routes.[103]

9-chloro-1-methoxyacridine (4): Yield: 8%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.05 (s, 3H), 6.87 (d, *J* = 7.6 Hz, 1H), 7.58-7.69 (m, 2H), 7.76-7.84 (m, 2H), 8.16 (d, *J* = 8.8 Hz, 1H), 8.59 (m, 1H).

9-chloro-3-methoxyacridine (5): Yield: 72%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.01 (s, 3H), 7.30 (dd, *J* = 9.4, 2.5 Hz, 1H), 7.44 (d, *J* = 2.3 Hz, 1H), 7.58 (m, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 8.31 (d, *J* = 9.1 Hz, 1H), 8.39 (d, *J* = 9.0 Hz, 1H).

9-chloro-1,3-dimethoxyacridine (6): Yield: 76%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.97 (s, 3H), 4.00 (s, 3H), 6.51 (d, *J* = 2.2 Hz, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 7.53 (m, 1H), 7.75 (m, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 8.51 (d, *J* = 8.8 Hz, 1H).

3-methoxy-N-decylacridin-9-amine (9): Yield: 83 %; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.6 Hz, 3H), 1.21-1.38 (m, 12H), 1.39-1.49 (m, 2H), 1.72-1.82 (m, 2H), 3.78 (t, *J* = 7.4 Hz, 2H), 3.97, s, 3H), 7.03 (dd, *J* = 9.5, 2.3 Hz, 1H), 7.30-7.37 (m, 2H), 7.66 (m, 1H), 7.98-8.05 (m, 3H).

N-(2-(1H-indol-3-yl)ethyl)-3-methoxyacridin-9-amine (10): Yield: 72%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.22 (t, *J* = 6.5 Hz, 2H), 3.94 (s, 3H), 4.17 (t, *J* = 6.6 Hz, 2H), 6.93 (dd, *J* = 9.5, 2.6 Hz, 1H), 7.09 (d, *J* = 2.2 Hz, 1H), 7.16 (m, 1H), 7.19-7.27 (m, 2H), 7.32 (d, *J* = 2.2 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.61 (t, *J* =

7.6 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.82-7.88 (m 2H), 8.00 (d, *J* = 8.0 Hz, 1H), 8.39 (br, s, 1H).

N-(1-benzylpiperidin-4-yl)-3-methoxyacridin-9-amine (11): Yield: 77%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.67-1.79 (m, 2H), 2.00-2.15 (m, 4H), 2.84-2.92 (m, 2H), 3.51 (s, 2H), 3.81 (br, s, 1H), 3.98 (s, 3H), 7.09 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.27-7.34 (m, 5H), 7.36-7.41 (m, 2H), 7.68 (t, *J* = 6.9 Hz, 1H), 7.97 (d, *J* = 9.6 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H).

9-(decylamino)acridin-3-ol (12): Yield: 44%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.83 (t, *J* = 6.8 Hz, 3H), 1.13-1.39 (m, 14H), 1.71-1.81 (m, 2H), 3.87 (t, *J* = 7.1 Hz, 2H), 6.78-6.88 (m, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 8.20 (d, *J* = 9.6 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 1H).

9-(2-(1*H*-indol-3-yl)ethylamino)acridin-3-ol (13): Yield: 35%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.13-3.18 (m, 2H), 4.05 (t, *J* = 7.5 Hz, 2H), 6.66 (br, s, 2H), 6.96 (m, 1H), 7.05 (m, 1H), 7.12-7.22 (m, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.50-7.60 (m, 3H), 8.07 (m, 1H), 8.24 (d *J* = 8.2 Hz, 1H), 10.83 (s, 1H).

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