University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2018

Synthesis and Evaluation of Modifications to the Central Ring of Depsidone Natural Products

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Abstract

Depsidones are a class of natural products that exhibit potent antibacterial activity (MIC = 0.0825-8 μg/mL) against vancomycin and methicillin resistant *staphylococcus aureus*. In order to increase the antibiotic potency of the depsidone family of natural products, modifications in size and connectivity are being explored. Although a synthesis for the core 6,7,6-fused tricyclic structure of depsidone exists, the yields of the final products are low. In an effort to optimize the yields, a new synthesis has been derived which involves Chan-Lam copper catalyzed coupling of diversified boronic acids and a substituted diol, amine hydroxyl, and thiol hydroxyl benzene rings followed by deprotection and esterification steps to close the central ring. Following this same synthesis, the core structure of depsidone was modified to change the ester linkage to both an amide and a thioester. These changes provided insight into the effect the electronic interactions have on antibiotic activity. All analogs synthesized have been evaluated in an antibacterial assay against Gram-positive and Gram-negative bacteria in order to build a structure activity relationship profile around depsidone core modifications.

1. Introduction

In 1904, Paul Ehrlich hypothesized that a certain compound could be found to specifically target invading and infectious microorganisms without harming the host cells, which initiated antimicrobial research. From that point, Ehrlich began to perform large-scale, systematic screenings to find a drug to treat syphilis by synthesizing hundreds of derivatives of the drug Atoxyl. These derivatives were tested for efficacy by injecting them into syphilis-infected rabbits and recording the progress of the infection. This process led to the discovery of the drug Salvarsan as an effective treatment for syphilis.[1]

Ehrlich's method was utilized by scientists at Bayer laboratories in the discovery of the family of antibiotics known as sulfonamides. This drug family proved to be both cost-effective and successful in the treatment of a broad spectrum of non-viral infections. Because of the economic and therapeutic implications, many companies began to mass produce sulfonamide derivatives. However due to the mass production and subsequent over prescription of sulfonamides, sulfonamide (sulfa) drug resistance, one of the most widespread cases of drug resistance, was seen shortly after the drug's introduction. Sulfa drug resistance proved to be just the beginning of the drug resistance problem that continues to occur today and encouraged the furtherance of antimicrobial research and development.[1,2]

In 1928, Alexander Fleming changed the field of medicine with his discovery of the antibiotic penicillin, the first known member of the β-lactam family of antibiotics. Fleming developed a screening method that used the inhibition zones of pathogenic bacteria grown on an agar medium plate to quantify the inhibitory effect of compounds. Aware of the resistance problem faced in the sulfa drug family, Fleming cautioned that using penicillin too much could lead to a similar outbreak of resistant bacteria. Fleming's caution was well-founded because β-lactam antibiotic resistant

strains of bacteria, like methicillin resistant *Staphylococcus aureus* (MRSA), affecting over one million patients in the United States alone annually.[1,3]

The growing public health issue of antibiotic resistance has encouraged a new push in the scientific community to develop novel antibiotics. Due to the urgency of the issue, scientists expedite the drug discovery process by analyzing natural products for antimicrobial activity. Once the product responsible for the activity is isolated, a viable synthetic scheme for that molecule is devised in a laboratory setting. The molecule is then modified in specific ways to probe and optimize the activity of the molecule via structure activity relationship studies. The data gained from these studies is important in elucidating the structure of the active site and the mechanism of action. Moreover, this data can be used to enhance the antibiotic activity of the molecule as well as increase its efficacy within the human body.[4]

One source of natural products are lichens, a fungus in a symbiotic relationship with green algae. In the 1940s, about fifty percent of the lichens tested showed some inhibitory effect on bacterial growth through extracted secondary metabolites. Following this discovery, researchers extracted and purified the compounds within lichens that were responsible for the antibiotic properties. One isolated compound, called depsidone, is biosynthesized within lichens and is responsible for its widespread medicinal properties.[5]

The depsidone class of natural products all contain a 6,7,6-fused ring system with varying substituents on the A and C ring (Figure 1). Two substituted phenyl groups are joined by an ester and ether linkage within a heptacyclic lactone ring, as seen in Figure 1. The functional groups on the A and C rings vary in nature and can be manipulated in a laboratory setting to alter the medicinal qualities of the compound. Depsidones have been shown to possess anticancer, anti-inflammatory, and antibacterial properties against both susceptible and resistant bacterial strains. The mechanism of action as to how the compounds kill the bacteria is still largely unknown. One study did subject analogs of depsidone isolated from soil bacteria to a permeability assay. No evidence of cell lysis was observed, which was indicative of an intracellular mechanism of action. Since their initial discovery, new, naturally-occurring depsidone analogs have been found in various lichens and deep-sea fungi. These analogs have been extracted and purified to further investigate which analogs have the highest inhibitory effects. This research has provided some insight into which substituents of depsidone provide the antimicrobial properties. The data allowed researchers to begin synthesizing new analogs in laboratories in an effort to improve depsidone as a means of developing a novel antibiotic.[5-9]

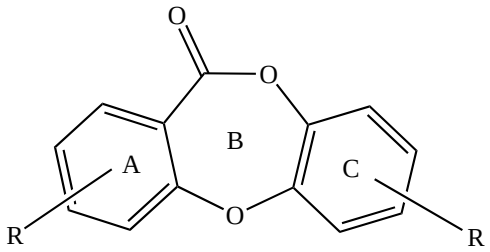


Figure 1: Core depsidone structure

Although usually exclusively found in lichen, new analogs of depsidones were found to be synthesized in other deep sea fungi. An article published in 2014 detailed the discovery of fifteen new analogs of depsidones. Nui and co-workers named these compounds Spiromastixone A-O and tested each compound against a panel of Gram-negative and Gram-positive bacteria. It was shown that there was no inhibition against Gram-negative bacteria but varying success in inhibiting Gram-positive bacteria with MIC values ranging from 0.125 to 8.0 μg/mL. Additionally, these new depsidone derivatives were tested against many multidrug-resistant Gram-negative and Gram-positive bacteria. Four of the analogs inhibited methicillin-resistant *Staphylococcus aureus*, resistant to β-lactam antibiotics and the cephalosporin family, better than the control, which yielded MIC values of 0.125 μg/mL. Spiromastixone I (Figure 2) successfully inhibited vancomycin-resistant *Enterococcus* species. Inhibitory effects of the analogs increased as the number of chlorine substituents increased.[8]

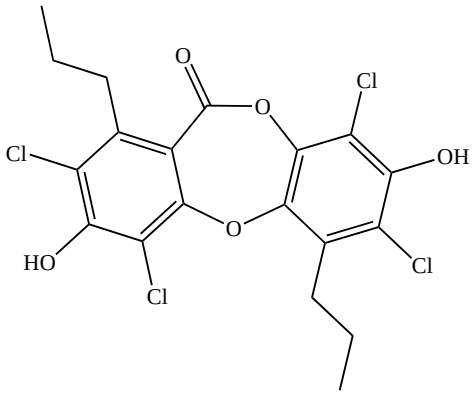
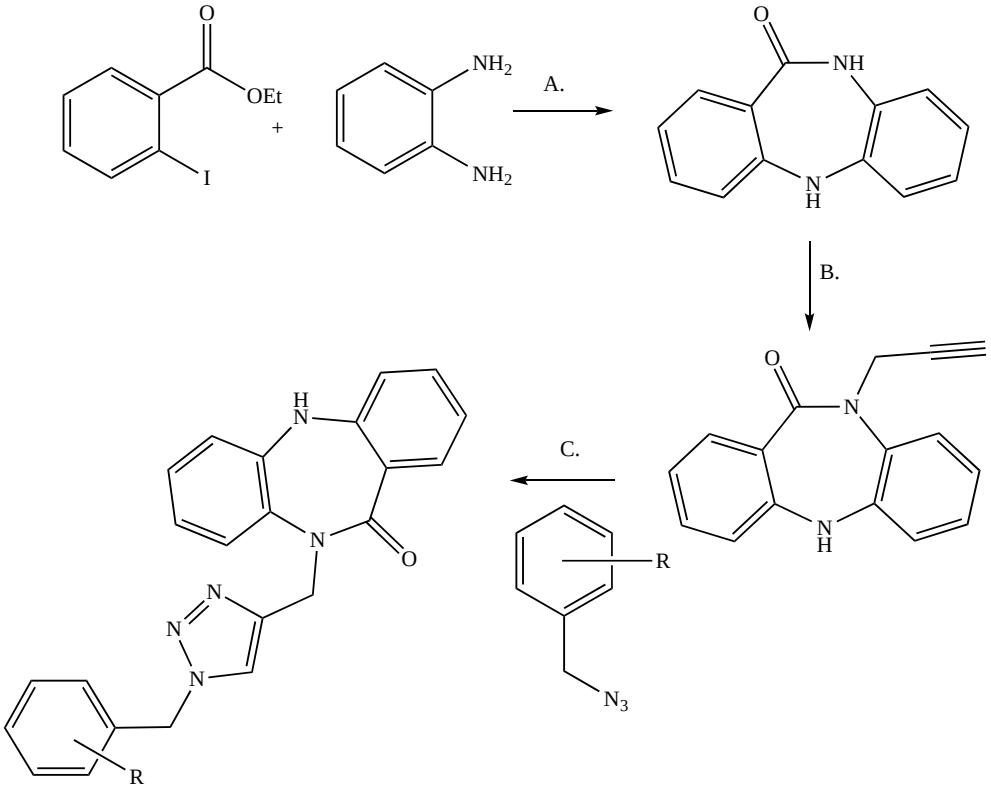


Figure 2: Spiromastixone I

Within the Kumar research group, analogs of depsidone with the ester and ether linkages of the β-lactam ring changed to amides and amines respectively, called dibenzodiazepinones (Figure 3), were tested for anticancer properties. To synthesize the desired analog, *o*-phenylenediamine and ethyl-2-iodobenzoate were reacted under inert conditions (Scheme 1). The anticancer screen revealed that the derivatives showed potency against certain breast cancer and prostate cancer cell lines. The results pointed to substituents in the *meta*-position to the ether linkage or an unsubstituted C ring as increasing the inhibitory effect.



Scheme 1: Kumar reaction synthesis scheme

A. CuI, K_3PO_4, ethylene glycol
B. *n*-BuLi, propargylbromide
C. $CuSO_4$, sodium ascorbate, *t*-BuOH/H_2O

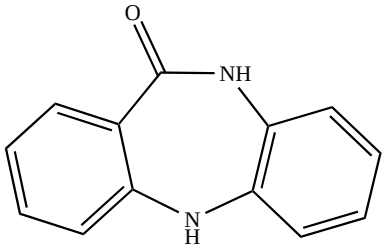


Figure 3: Dibenzodiazepinone

The same group of analogs of *N*-(2-hydroxyphenyl)anthranisic acid were also tested for anti-inflammatory characteristics. These analogs replaced one ether linkage in the B ring with an amine, as seen in Figure 4. The Raines research group prepared the anthranisic acid from *o*-bromo or *o*-chlorobenzoic acid and substituted *o*-amino phenol by an Ullmann-type condensation reaction. Dicyclohexylcarbodiimide was used to close the central ring. The analog was shown to possess anti-inflammatory properties.[11]

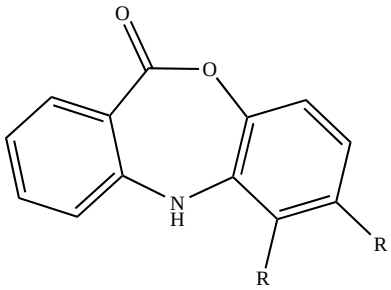


Figure 4: Dibenz[b,e][1,4]oxazepin-11(5H)-one

Within the Wolfe research group, initial research has been accomplished over the past three years toward development of depsidone analogs. A previous member of the research group, John Terrell, designed a reliable synthesis for an unsubstituted depsidone, largely based on coupling through the ether initially as outlined by the Deraeve group. Terrell began to research how changing the ester linkage of the B ring to an amine and an amide affected the antibacterial activity of the depsidone.[12] Another past research member, Joshua Katz, focused on changing the substituents on the A and C rings of depsidone. Both of these projects had the aim of finding common substituents or similar analogs that increase the antibiotic activity of the depsidone.[14]

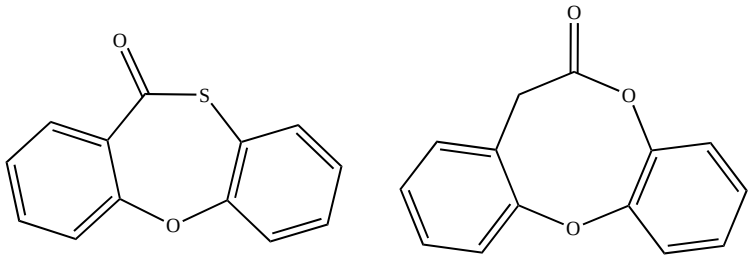


Figure 5: Terrell analogs

In all previous research, many of the analogs were synthesized in similar manners. Two substituted phenyl rings are joined together via copper(I) iodide, potassium permanganate, or various other reagents. Depending on the identity of the desired product, the substituents of the reactants will change accordingly. The method by which the ring is closed changes as the substituents change or the size of the B ring changes in the desired final product. [6,10,11,14,17]

Herein, a new, more reliable synthesis of modified depsidones is described, which was used to begin exploring how variations of the B ring affect the antibiotic efficacy of depsidone. Most of the published research about B ring alterations has involved the modification of the ester linkage to amines or amides. To further advance the depsidone research, the ester linkages and the carbonyl atom were altered (Figure 6). Once synthesized, the analogs were tested for antibiotic inhibitory activity against Gram-negative and Gram-positive bacteria. The quantitative data from the assays were used to extrapolate the inhibitory concentration of fifty percent (IC_{50}) which measures the antibiotic strength of the analogs. From this data, how modifications of the A and B rings affect the antibacterial properties of depsidone were elucidated. A preliminary structure activity relationship profile was made in order to potentially improve the antibiotic research and offer insight into the molecule's mechanism of action and its active site.

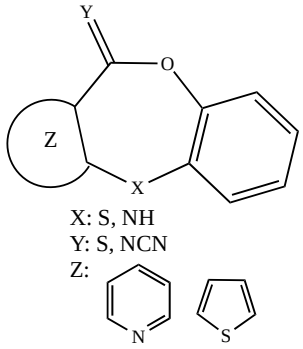


Figure 6: Initial analog targets

2. Experimental Methodology

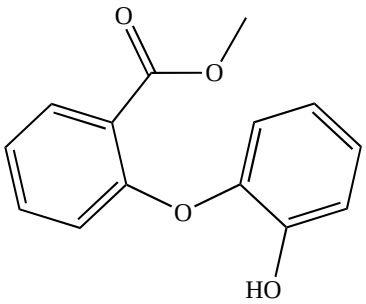
NMR: All spectra were obtained using $CDCl_3$ as the solvent on a Varian Gemini 2000 with an Oxford Instruments 400M Hz superconducting magnet.

LCMS: All spectra were obtained using CH_3OH as the solvent on Shimadzu LCMS-2020 using a 30-90 biogradient water-methanol and run for 7 min.

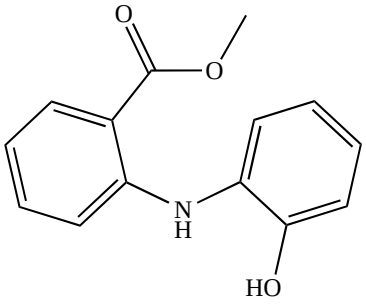
Reagents: All reagents were purchased from commercial sources as either reagent grade or anhydrous and used without prior modifications, except for anhydrous solvents dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF), which were dried prior to use in a dry and inert solvent system. Reactions carried out under inert conditions were run in flame or oven dried glassware that had been purged of oxygen and backfilled with Ar(g).

2.1. General Procedure 1 for Esterification Using Boronic Acid.

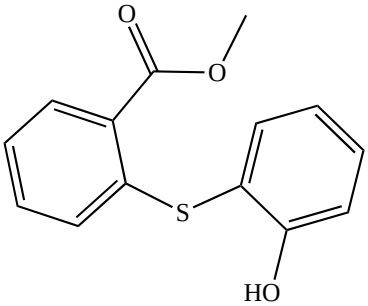
The boronic acid was combined with a catechol derivative in copper acetate, pyridine, and CH_2Cl_2 in a 3:1:2:3 molar ratio and stirred for 24 h at room temperature. Upon completion, the product was filtered through Celite using CH_2Cl_2 to wash. The crude mixture was separated via flash column chromatography (SiO_2, 10% ethyl acetate, 90% hexane).



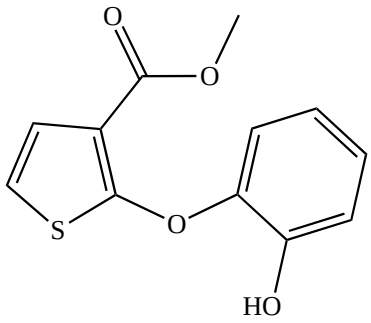
Methyl 2-(2-hydroxylphenyl)benzoate (A) Boronic acid (500-515 mg, 2.78-2.86 mmol) was combined with catechol (100-114 mg, 0.93-1.04 mmol) in copper acetate (336 mg, 1.85 mmol), pyridine (0.22-0.25 mL, 2.73-2.98 mmol), and CH_2Cl_2 (2.4 -5.0 mL, 39.0-78.0 mmol) following **General Procedure 1** to give **A** (173–114.1 mg, 51-76%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 8.027 (d, *J*=7.6, 1H), 7.547 (t, *J*=8.8, 1H), 7.442 (m, 1H), 7.226 (d, *J*=8 1H), 7.148 (m, 1H), 3.629 (s, 3H) ppm. **LCMS:** M +1, 245; M-1, 243, 2.7 min.



Methyl 2-((2-hydroxyphenyl)amino)benzoate (D) Boronic acid (2.47 g, 4.58 mmol) was combined with 2-aminophenol (501 mg, 13.7 mmol) in copper acetate (1.66 g, 9.14 mmol), pyridine (1.07 mL, 13.3 mmol), and CH_2Cl_2 (25 mL, 385 mmol) following **General Procedure 1** to give **D** as an orange solid (122.2 mg, 7-10%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 7.997 (d, *J*=15.2, 1H), 7.523 (m, 1H), 7.409 (m, 1H), 7.193 (d, *J*=7.6, 1H), 3.591, (s, 3H), 2.142 (s, 3H) ppm. **LCMS:** M +1, 244; M-1, 242, 0.6 min.



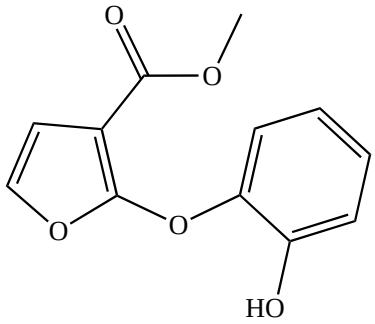
Methyl 2-((2-hydroxyphenyl)thio)benzoate (G) Boronic acid (1.43 g, 7.94 mmol) was combined with 2-mercaptophenol (0.63 mL, 3.96 mmol) in copper acetate (1.44 g, 7.95 mmol), pyridine (957 mL, 11.9 mol), and CH_2Cl_2 (24 mL, 385 mmol) following **General Procedure 1** to give **G** as an pink solid (946.2 mg, 96%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 8.030 (d, *J*=8, 1H), 7.531 (d, *J*=8, 1H), 7.442 (t, *J*=8.4, 1H), 7.292 (t, *J*=8.8, 1H), 7.189 (t, *J*=8, 1H), 7.115 (d, *J*=8, 1H), 7.014 (t, *J*=7.2, 1H), 6.724 (d, *J*=7.6, 1H), 6.615 (s, 1H) 3.997 (s, 3H) ppm. **LCMS:** M +1, 249; M-1, 247, 0.4 min.



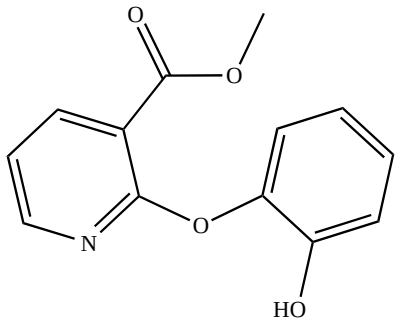
Methyl 2-(2-hydroxyphenoxy)thiophene-3-carboxylate (T) The heterocycle boronic acid **(S)** (3.69g, 19.9 mmol) was combined with catechol (1.1g, 9.9 mmol) in copper acetate (3.6 g, 19.9 mmol), pyridine (2.4 mL, 29.8 mmol), and CH_2Cl_2 (148 mL, 2.3 mol) following **General Procedure 1** to give **S** (87.2 mg, 4%) a greasy light brown as the product. **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 7.327 (d, *J*=0.8, 1H), 6.974 (d, *J*=0.8, 1H), 6.877 (m, J=4, 2H), 6.792 (m, *J*=2 ,2H), 7.148 (m, 1H), 3.533 (m, J=2, 3H) ppm. **LCMS:** M +1, 251; M-1, 249, 2.85 min.

2.1.1. general procedure 1a for esterification using boronic acid.

The boronic acid was combined with a catechol derivative in copper acetate, anhydrous pyridine, and CH_2Cl_2 in a 3:1:1:3 molar ratio and stirred for 24 h at room temperature. Upon completion, the product was filtered through Celite and a fritted funnel using ethyl acetate to wash. The filtered crude was then spun with ethylenediaminetetraacetic acid (EDTA) and saturated aqueous $NaHCO_3$ for 20 minutes. The EDTA and copper acetate were then removed via extracted using ethyl acetate and water. The organic layer was collected, dried over Na_2SO_4, filtered, and condensed under reduced pressure. The crude mixture was separated via flash column chromatography (SiO_2, 10% ethyl acetate, 90% hexane).



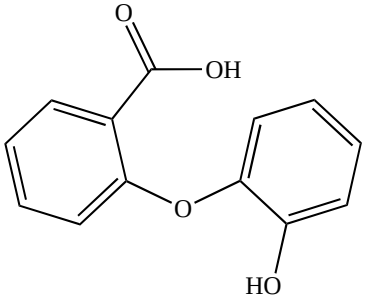
methyl 2-(2-hydroxyphenoxy)furan-3-carboxylate (W) Carboxylic acid derivative (500 mg, 2.9 mmol) **P**, was combined with catechol (162 mg, 1.5 mmol) in copper acetate (266 mg, 1.5 mmol), pyridine (0.4 mL, 4.4 mmol), and CH_2Cl_2 (20 mL) following **General Procedure 1a** to give **V** (87.2 mg, 4%) a brown solid as the product



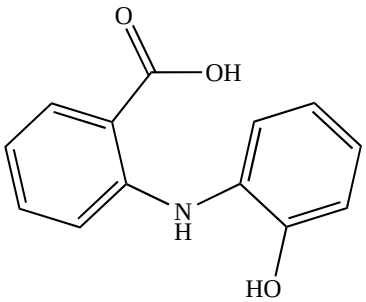
Methyl 2-(2-hydroxyphenoxy)nicotinate (X) Carboxylic acid derivative (500 mg, 2.8 mmol) **N**, was combined with catechol (152 mg, 1.4 mmol) in copper acetate (251 mg, 1.4 mmol), pyridine (0.3 mL, 4.2 mmol), and CH_2Cl_2 (20 mL) following **General Procedure 1a** to give **X** as a dark orange solid (148 mg, 4%) a brown solid as the product. **LCMS:** M +1, 246; M-1, 224, 4.9 min.

2.2. General Procedure 2 for Deprotection of Methyl Ester

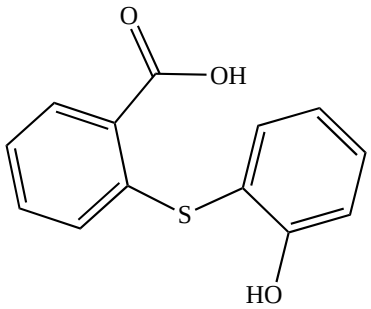
The ester coupled products were dissolved in aqueous NaOH (2M) and CH_3OH (0.1M) and stirred for 24 h at room temperature in a 1:530:1 molar ratio. Once completed, the crude reaction was acidified to pH 4 with aqueous HCl (1N) and extracted with ethyl acetate (1x). The organic layer was then washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated using reduced pressure.



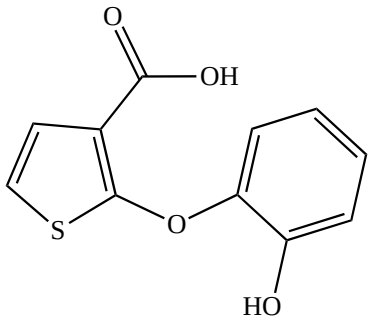
2-(2-hydroxyphenyl)benzoic acid (B) Benzoate (173–114.1 mg, 0.47-0.71 mmol) **A,** NaOH (2M, 4.7-7.1 mL, 248-377 mmol), and CH_3OH (0.1M, 4.7-7.1 mL) following **General Procedure 2** to give **B** (67.7-88.1 mg, 41-81%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 8.092 (d, *J*=8.8, 1H), 8.002 (d, *J*=8, 2H), 7.592 (t, *J*=8.8, 1H) 7.485 (m, 3H), 7.175 (m, 3H), 7.153, (d, *J*=9.2, 4H), 7.070 (m, 4H), 6.905 (t, *J*=9.2, 2H) ppm. **LCMS:** M +1, 23; M-1, 229, 1.95 min.



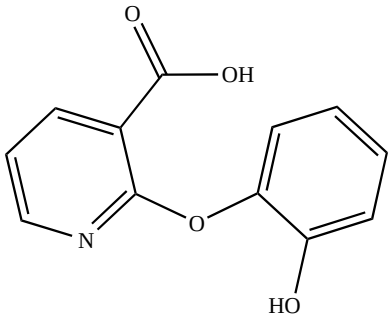
2-((2-hydroxyphenyl)amino)benzoic acid (E) Benzoate (122.2 mg, 0.50 mmol) **D,** NaOH (2M, 5.0 mL, 267 mmol), and CH_3OH (0.1M, 5.0 mL, 124 mmol) following **General Procedure 2** to give **E** as a light pink solid (83.6 mg, 72%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 8.060 (d, *J*=8, 2H), 7.603 (t, *J*=6, 2H), 7.457 (t, *J*=8, 2H), 7.240 (d, *J*=7.6, 1H) ppm. **LCMS:** M +1, 230; M-1, 228, 0.8 min.



2-((2-hydroxyphenyl)thio)benzoic acid (H) Benzoate (946.2 mg, 3.7 mmol) **G,** NaOH (2M, 36.8 mL, 73.64 mmol), and CH_3OH (0.1M, 36.8 mL, 36.82 mmol) following **General Procedure 2** to give **H,** a yellow solid.(806.3 mg, 94%). **^{1}H-NMR:** ($CDCl_3$-*d*, 400 MHz) δ 8.181 (d, *J*=7.6, 1H), 7.536 (d, *J*=8, 1H), 7.461 (t, *J*=15.2, 1H), 7.341 (t, *J*=8, 1H), 7.263 (t, *J*=7.6, 2H), 7.131 (d, *J*=8, 1H), 7.030 (t, *J*=7.6, 1H), 6.741 (d, *J*=8, 1H), 6.650 (s, 1H) ppm. **LCMS:** M +1, 235; M-1, 233, 0.37 min.



2-(2-hydroxyphenoxy)thiophene-3-carboxylic acid (V) Carboxylate (87.2 mg, 0.35 mmol) **U,** NaOH (2M, 3.5 mL), and CH_3OH (0.1M, 3.5 mL) following **General Procedure 2** to give **T** (57.6 mg, 70%) as a dark brown liquid. **^{1}H-NMR:** inconclusive. **LCMS:** M +1, 237; M-1, 235, 4.1 min.

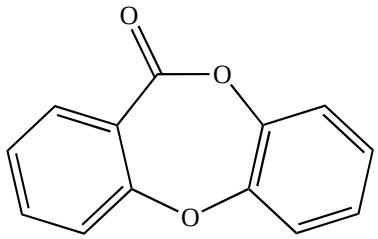


2-(2-hydroxyphenoxy)nicotinic acid (Y) Carboxylate (15 mg, 0.06 mmol) **X,** NaOH (2M, 2.4 mL), and CH_3OH (0.1M, 2.4 mL) following **General Procedure 2** to give **Y** (12 mg, 87%) as a dark orange solid. **LCMS:** M +1, 231; M-1, 229, 0.6, 1.1 min.

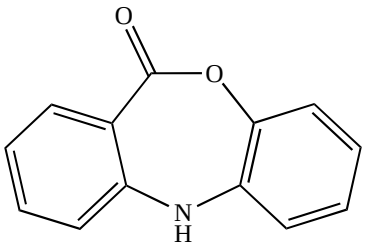
2.3. General Procedure 3 for Intramolecular Esterification for Ring Closure

The carboxylic acid was dissolved in anhydrous THF (20 mM) and combined with anhydrous pyridine and thionyl chloride in a 1:20:3 molar ratio in a flame-dried round bottom flask under an inert Ar atmosphere and stirred for 24 h at room temperature. The crude reaction was acidified using aqueous HCl (1N) and extracted using ethyl acetate (1x). The organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over

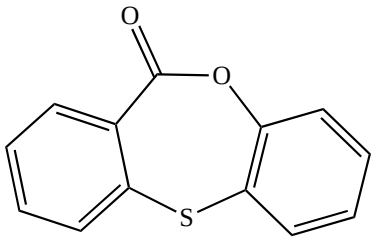
anhydrous Na_2SO_4, and concentrated using reduced pressure. The crude products were separated using flash column chromatography (SiO_2, 10% ethyl acetate, 90% hexane).



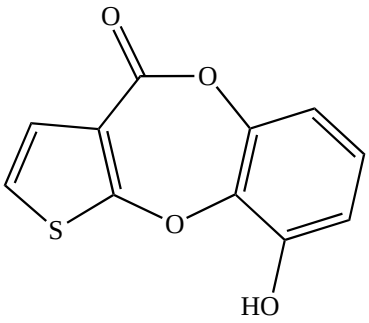
11*H*-dibenzo[*b,e*]dioxepin-11-one (C) Benzoic acid (67.7-88.1 mg, 0.29-0.38 mmol) **B**, pyridine (0.47-.62 mL, 5.88-7.65 mmol), and thionyl chloride (0.06 mL, 0.88 mmol) were combined according to **General Procedure 3** to give **C** as a brown solid (6.8–15.8 mg, 10-19%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 7.973 (d, *J*=7.2, 1H), 7.576 (t, *J*=9.2, 1H), 7.268 (m, 4H), 7.183 (m, 2H) ppm. **LCMS:** M +1, 213; M-1, 211, 6.1 min.



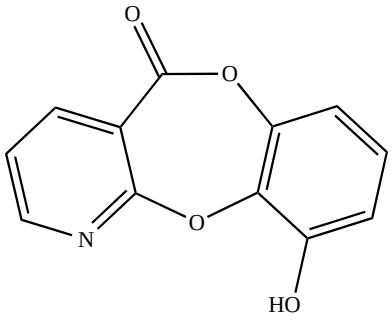
Dibenzo[*b,e*][1.4]oxazepin-11(5*H*)-one (F) Benzoic acid (83.6 mg, 0.37 mmol) **E**, pyridine (0.59 mL, 72.9 mmol), and thionyl chloride (0.079 mL, 1.09 mmol) were combined according to **General Procedure 3** to give **F** as a brown greasy product**,** (43.5 mg, 56 %). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 8.309 (d, *J*=6.8, 1H), 7.66 (t, *J*=7.6, 1H), 7.576 (m, 1H), 7.227 (d, *J*=7.2, 1H) ppm. **LCMS:** M +1, 212; M-1, 210, 0.5 min.



11*H*-dibenzo[*b,e*][1,4]oxathiepin-11-one (J) Benzoic cid (806.3 mg, 1.2 mmol) **H**, pyridine (5.57 mL, 76.8 mmol), and thionyl chloride (0.75 mL, 9.3 mmol) were combined according to **General Procedure 3** to give **J** as a product**,** (9.4 mg, 1%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) inconclusive. **LCMS:** M +1, 229; M-1, 227, 0.4 min.



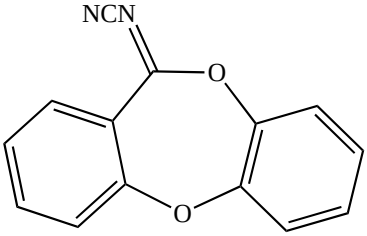
9-hydroxy-4*H*-benzo[*b*]thieno[2,3-*e*][1,4]dioxepin-4-one (U) Product **T** (57.6 mg, 0.244 mmol), pyridine (0.4 mL, 4.88 mmol), and thionyl chloride (0.05 mL, 0.87 mmol) were combined according to **General Procedure 3** to give **T** as a yellow greasy product (36.9 mg, 69%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz) δ 6.888 (d, *J*=5.2, 2H), 6.944 (m, *J*=4.8, 1H), 7.241 (d, *J*=4.4, 2H), 7.283 (d of d, *J*=3.2, 1H) ppm. **LCMS:** M +1, 219; M-1, 217, 3.4, 1.6 min.



10-hydroxy-5H-benzo[2,3][1,4]dioxepino[5,6-b]pyridin-5-one (Z) Product **Y** (12 mg, 0.05 mmol), pyridine (0.08 mL, 1.0 mmol), and thionyl chloride (0.1 mL, 0.15 mmol) were combined according to **General Procedure 3** to give **Z** as a white solid (9 mg, 85%).

2.4. General Procedure 4 for Cyanoimidation

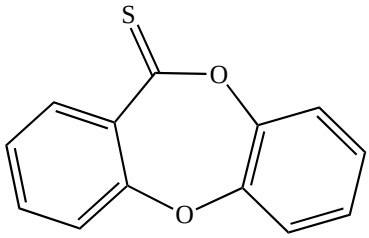
The unmodified core was dissolved in anhydrous methanol (8M) and combined with potassium t*ert*-butoxide and cyanamide in a 1:4:4 molar ratio. The reaction was covered and stirred at room temperature for 0.5 hr. *N*-bromosuccinimide was then added to the reaction at a 1:4 molar ratio relative to the depsidone core. The reaction was refluxed at 50 ºC for 11 hours. The crude reaction was extracted using H_2O and ethyl acetate. The organic layer was collected and washed with saturated aqueous NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated using reduced pressure. The crude products were separated using flash column chromatography (SiO_2, 20% ethyl acetate, 80% hexane).



***N*-(11*H*-dibenzo[*b,e*][1,4]dioxepin-11-ylidene)cyanamide (K)** Benzodioxipinone (403.9 mg, 1.9 mmol), **C**, methanol (15.2 mL, 375 mmol), cyanamide (320 mg, 7.6 mmol), potassium tert-butoxide (854 mg, 7.6 mmol), and *N*-bromosuccinimide (1355 mg, 7.6 mmol) were combined according to **General Procedure 4** to give **K** as a brown solid, (343.7 mg, 81%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz). **LCMS:** M +1, 225; M-1, 223, 2.65, 1.75 min.

2.5. General Procedure 5 for Exchange of Ketone for Thioketone

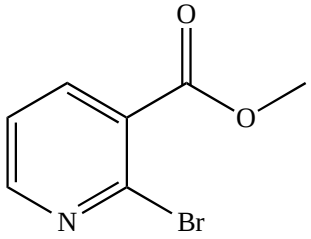
The unmodified depsidone core was dissolved using anhydrous toluene (0.15M) and added to Lawesson's reagent in a 20:13 molar ratio before being transferred into a flame-dried round bottom flask. The reaction was refluxed at 110 ºC in the dark for 20 hours under inert conditions. The reaction was cooled to room temperature before undergoing concentration under reduced pressure. The crude products were separated using flash column chromatography (SiO_2, 5% ethyl acetate, 95% hexane).



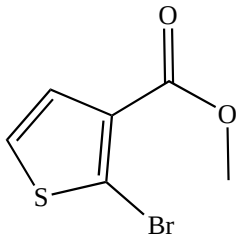
11*H*-dibenzo[*b,e*][1,4]dioxepine-11-thione (L) Benzodioxipinone (36.3 mg, 0.171 mmol), **C**, Lawesson's reagent (44.9 mg, 0.111 mmol), and toluene (1.6 ml, 15.1 mmol) were combined according to **General Procedure 5** to give **L** as a yellow product.

2.6. General Procedure 6 for Methylating Carboxylic Acid Heterocycles

The carboxylic acid heterocycle was added to methanol and sulfuric acid in a 2:2:1 molar ratio. The reaction was refluxed for 9 hours at 65 ºC under argon. The organic layer was extracted using saturated aqueous $NaHCO_3$ and ethyl acetate (2x). The organic layers were collected, dried over anhydrous $NaSO_4$, filtered, and condensed under reduced pressure. The desired product was separated using flash column chromatography (SiO_2, 10% ethyl acetate, 90% hexane).



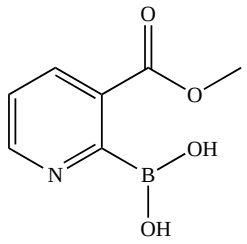
Methyl 2-bromonicotinate (M) 2-bromonicotinic acid (2.0 g, 9.9 mmol), methanol (10.0 mL, 9.9 mmol), and sulfuric acid (0.438 mL, 4.95 mmol) were combined according to **General Procedure 6** to give **M** as a brown solid (1.14 g, 21%). **^{1}H-NMR:** ($CDCl_3$-d_1, 400 MHz)) δ 8.451 (s, *J*=8, 1H), 8.067 (d, *J*=11.2, 1H), 7.343 (t, *J*=13.6, 1H), 3.931 (s, *J*=3.2, 3H) ppm.



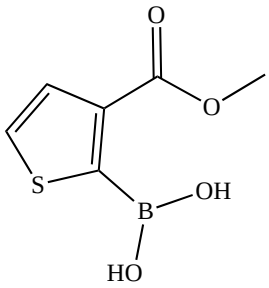
Methyl-2-bromothiophene-3-carboxylate (O) 2-bromothiophene-3-carboxylic acid (10.0 g, 483 mmol), methanol (50 mL, 1.23 mol), and sulfuric acid (1.3 mL, 49.8 mmol) were combined according to **General Procedure 6** to give **O** as a brown product.

2.7. General Procedure 7 for Borating the Methylated Heterocycles

The methylated heterocycle was combined with magnesium turnings and lithium chloride in anhydrous THF (0.5 M) in a 4:10:5 molar ratio with 4 Å molecular sieves in a flame-dried round bottom flask. The reaction was then allowed to stir at room temperature for 9-11 hours under inert conditions. The reaction was placed in an ice bath and trimethylborate was added in a 1:2 molar ratio relative to the heterocycle. The reaction was stirred in an ice bath for four hours in inert conditions. The reaction was acidified using 1N HCl. The organic layer was extracted using ethyl acetate and 1N HCl. The organic layer was dried using anhydrous Na_2SO_4, filtered, and condensed using reduced pressure to yield the desired product.



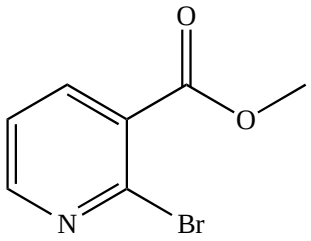
(3-(methoxycarbonyl)pyridin-2-yl)boronic acid (N) Methylated nitrogenous heterocycle (1.1425 g, 5.29 mmol), **M**, magnesium turnings, (317 mg, 13.2 mmol), 0.5 M lithium chloride in THF (13.22 mL, 6.61 mmol), and trimethylborate (1.18 mL, 10.6 mmol) were combined according to **General Procedure 7** to give **N** as a brown product (510.6 mg, 53%).



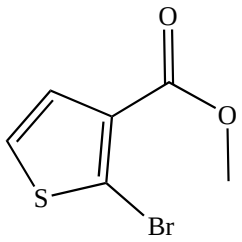
(3-(methoxycarbonyl)thiophen-2-yl)boronic acid (Q) Methylated thiophene heterocycle (5.0 g, 22.6 mmol), **O**, magnesium turnings, (1.4 mg, 56.5 mmol), 0.5 M lithium chloride in THF (56.5 mL, 28.5 mmol), and trimethylborate (5.0 mL, 45.2 mmol) were combined according to **General Procedure 7** to give **Q** as a silty crude product.

2.8. General Procedure 8 for Methylating Heterocylces using Activated Acid

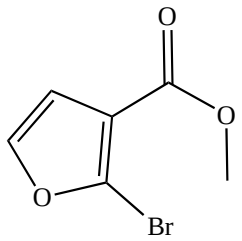
The carboxylic acid heterocycle was added to thionyl chloride in a 3:1 molar ratio into a flame-dried round bottom flask and dissolved in CH_2Cl_2 (0.5 M). The reaction was them warmed to reflux under inert conditions for 2 hours. The flask was placed into a dry ice/acetone bath and triethyl amine and anhydrous methanol were slowly added in a 1:10:10 molar ratio relative to the heterocycle. Upon completion of the addition the reaction was extracted using aqueous bicarbonate and ethyl acetate (2x). The organic layers were combined, dried over anhydrous $NaSO_4$, filtered, and condensed under reduced pressure. The crude product was carried on to the next reaction.



Methyl 2-bromonicotinate (M) 2-bromonicotinic acid (5 g, 24.8 mmol), thionyl chloride (9.0 mL, 74.3 mmol), methanol (10.1mL, 248 mmol), and triethylamine (34.7 mL, 248 mmol) were combined according to **General Procedure 8** to give **M** as a brown silty solid as the crude.



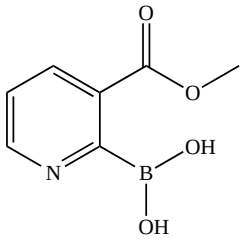
Methyl-2-bromothiophene-3-carboxylate (O) 2-bromothiophene-3-carboxylic acid (5.0 g, 24.2 mmol), thionyl chloride (8.8 mL, 72.4 mmol), methanol (9.8 mL, 241 mmol), and triethylamine (33.8 mL, 241 mmol) were combined according to **General Procedure 8** to give **O** as a cream silty solid as the crude.



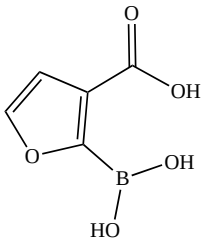
Methyl 2-bromofuran-3-carboxylate (P) 2-bromofuran-3-carboxylic acid (1.0 g, 5.24 mmol), thionyl chloride (1.9 mL, 15.7 mmol), methanol (1.0 mL, 25.4 mmol), and trimethylamine (3.6 mL, 25.4mmol) were combined according to **General Procedure 8** to give **P** as a cream silty solid as the crude.

2.9. General Procedure 9 for Boronating Heterocylces at Increased Temperature

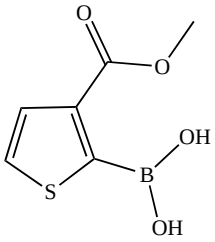
The methylated heterocycle were added to combined with magnesium turnings and lithium chloride in anhydrous THF (0.5 M) in a 4:10:5 molar ratio with 4 Å molecular sieves in a flame-dried round bottom flask. The reaction was stirred at room temperature for 2 hours under inert conditions. Trimethylborate was added in a 1:2 molar ratio relative to the heterocycle. The reaction was then allowed to continue to reaction for 5 hours under inert conditions. The reaction was acidified using 1N HCl and extracted with ethyl acetate and 1N HCl. The organic layer was dried using anhydrous Na_2SO_4, filtered, and condensed using reduced pressure to yield the desired product



(3-(methoxycarbonyl)pyridin-2-yl)boronic acid (N) Methylated nitrogenous heterocycle (5.0 g, 23.1 mmol), **M**, magnesium powder, (1.4 mg, 57.9 mmol), 0.5 M lithium chloride in THF (57.8 mL, 28.9 mmol), and trimethylborate (4.8 mL, 46.2 mmol) were combined according to **General Procedure 9** to give **N** as a brown crystal crude mixture. **LCMS:** M +1, 182; M-1, 180, 1.0 min.



2-boronofuran-3-carboxylic acid (R) Methylated furan heterocycle (1.0 g, 4.89 mmol), **P**, magnesium powder, (290 mg, 12.2 mmol), 0.5 M lithium chloride in THF (12.25 mL, 6.1mmol), and trimethylborate (1.1 mL, 9.78 mmol) were combined according to **General Procedure 9** to give **R** as a brown cream powder crude mixture. **LCMS:** M +1, 171; M-1, 169, 0.75 min.



2-boronofuran-3-carboxylic acid (S) Methylated furan heterocycle (1.0 g, 4.89 mmol), **P**, magnesium powder, (290 mg, 12.2 mmol), 0.5 M lithium chloride in THF (12.25 mL, 6.1mmol), and trimethylborate (1.1 mL, 9.78 mmol) were combined according to **General Procedure 9** to give **R** as a brown cream powder crude mixture. **LCMS:** M +1, 187; M-1, 185, 0.49 min.

2.10. General Procedure 10 for Sterile Conditions

The workbench and gloves were sterilized by washing with ethanol. All work under sterile conditions was completed under flame from a propane torch. Lids for sterile containers were flamed before replacing them on the container.

2.11. General Procedure 11 for Testing Compounds via 96-Well Plate Bioassay

The compounds to be tested were diluted in DMSO to 100 mg/mL with a final volume of at least 20 µL. Under sterile conditions following **General Procedure 10**, approximately 4 mL of full strength tryptic soy broth (FSTSB) were transferred into test tubes using an Eppendorf pipette. To one of the test tubes, a single colony of the bacteria of choice was added. The other test tube contained only FSTSB to ensure there was no contamination present. The bacterial colonies were grown up by incubating while shaking the test tubes for 18 hours at 37 ºC.

The assay plate was prepared under sterile conditions following **General Procedure 10**. The master plate was prepared by placing 9 µL of DMSO into each of the 96 wells using a micropipette except for top row (row A). The

top row contained 10 µL of one of the following: a) the compound being studied dissolved in DMSO, b) pure DMSO as the negative control, or c) the antibiotic chloramphenicol diluted to 100 mg/mL as the positive control. The tenfold dilution was completed by taking 1 µL from the top well and mixing it into the well below down each column. Test plates contained 10% of the overnight culture in FSTSB (99 µL total) and 1% of the test compound in DMSO (1 µL). Compounds were transferred from the master plate to the test plate. The plates were incubated with shaking for 18 hours at 37 ºC. Absorbance was read for each well on a BioTek plate-reader at 590 nm using Standard S. Aureus Assay Protocall_590nm_blanks.prt and analyzed using Gen5 2.09 software data package.

Bioassay Testing Compounds C, F, and J against *Staphylococcus Aureus* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-positive bacteria *Staphylococcus aureus* following **General Procedure 11**. Antibiotic activity was seen for compound **C** at the highest concentration.

Bioassay Testing Compounds C, F, and J against *Escherichia Coli* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-negative bacteria *Escherichia coli* following **General Procedure 11**. No antibiotic activity was seen for any of the compounds.

Bioassay Testing Compounds C and K against *Staphylococcus Aureus* Under sterile conditions following **General Procedure 10**, compounds **C and K** were tested against Gram-positive bacteria *Staphylococcus aureus* following **General Procedure 11**. Antibiotic activity was seen for K at high concentrations.

Bioassay Testing Compounds C and K against *Escherichia Coli* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-negative bacteria *Escherichia coli* following **General Procedure 11**. Antibiotic activity was seen for compound at high concentrations. Compound K exhibited antibiotic activity at high concentrations.

Bioassay Testing Compounds C, F, J, U, and K against *Staphylococcus Aureus* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-positive bacteria *Staphylococcus aureus* following **General Procedure 11**. Antibiotic activity was seen for compound **C** at the highest concentration, compound **U** in the first four concentrations, and possible activity for compounds **F, J,** and **K** at the highest concentration.

Bioassay Testing Compounds C, F, J, U, and K against *Escherichia Coli* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-negative bacteria *Escherichia coli* following **General Procedure 11**. Antibiotic activity was seen for compound **C** at the highest concentration.

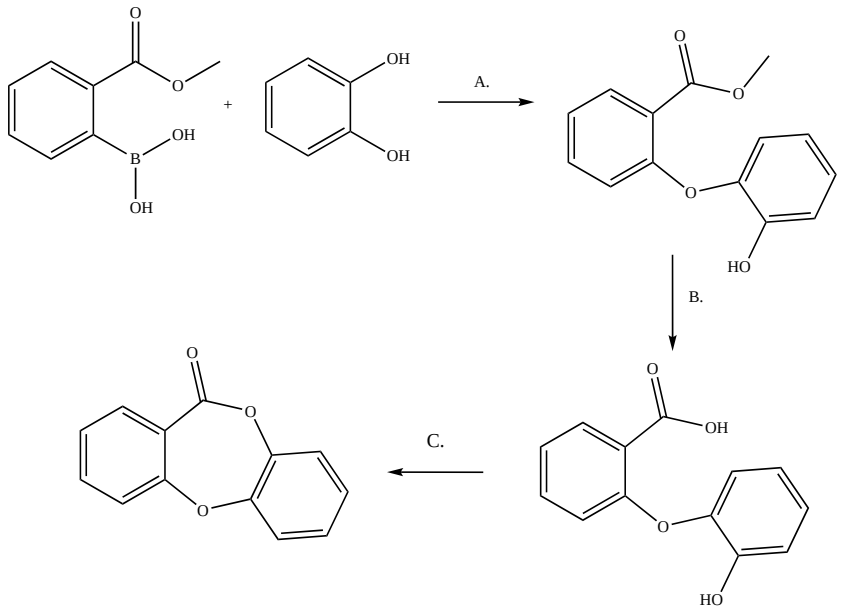
Bioassay Testing Compounds L, K, and Z against *Staphylococcus Aureus* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-positive bacteria *Staphylococcus aureus* following **General Procedure 11**. Visible, but not quantifiable, activity was seen for compound **L** at the highest concentrations.

Bioassay Testing Compounds L, K, and Z against *Escherichia Coli* Under sterile conditions following **General Procedure 10**, compounds **C, F, and J** were tested against Gram-negative bacteria *Escherichia coli* following **General Procedure 11**. No antibiotic activity was seen.

3. Results and Discussion

3.1. Optimizing the Core Synthesis

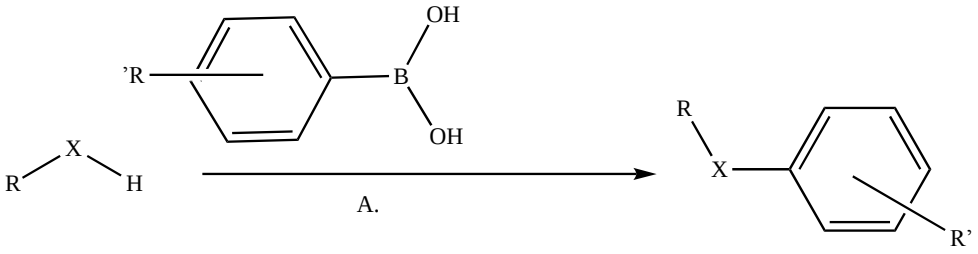
In accordance with the goals set forth at the beginning of the project, a reliable synthesis has been derived for the unmodified core structure of depsidone, **C**, (Scheme 2).



Scheme 2: Unmodified core synthesis scheme

A. $Cu(OAc)_2$, pyridine, CH_2Cl_2, r.t.
B. 2 M NaOH, 0.1 CH_3OH, r.t.
C. 20 mM THF, pyridine, thionyl chloride, inert, r.t.

ather than relying on previously published synthetic schemes for depsidone analogs, a method known as Chan-Lam coupling was used. Chan-Lam coupling is a method of arylating amines or alcohols using phenylboronic acid and cupric acetate, as an improvement on Ullmann-type condensations. This scheme (Scheme 3) is effective for producing heteroatom-carbon bonds that were useful in coupling the heterocycles used in to make the depsidone core.[21]



Scheme 3: Chan-Lam coupling synthesis scheme

A. CH_2Cl_2, r.t.
Et_3N or pyridine
X = N,O

Utilizing a commercially available boronic acid as one of the reagents eliminates the need for an additional protection step. The protection of the carboxyl is necessary in order to generate the ester linkage between the two phenyl groups, **A**. Although unnecessary with unsubstituted catechol, this step proves beneficial in the synthesis for modification of the ester linkages.

3.2. Synthesis of Ether Analogs

When attempting to make the first analog of depsidone**, E**, an aminophenol was used in place of the catechol in hopes of modifying the ester linkage on carbon 6 of the heptacyclic B ring. Synthetic scheme 3 was followed. Based on ^{1}H-NMR data for the products of steps 1 and 2, rotamers were present where the ring was rotating about the newly formed amine linkage. Additionally, a side product where the amine linkage was created at carbon 3 of the B ring, seen in Figure 7, was a possibility due to the non-specificity of the initial coupling step. Using the same synthetic scheme, additional analogs can be made by simply substituting one or both alcohol groups on the catechol for the desired final linkage groups. These analogs are valuable in probing how electronegativity can affect the binding to the active site. As the mechanism of action for depsidones is unknown, any structure activity relationship data gained is significant in attempting to elucidate the molecule's mechanism whereby its activity is derived.

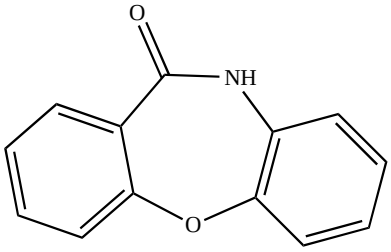
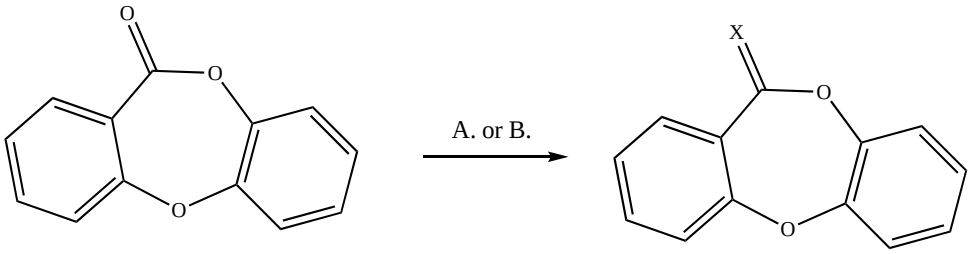


Figure 7: Side product of Scheme 3

3.3. Synthesis of Ketone Analogs

The second set of analogs exchanged the carbonyl atom for either a sulfur, **L**, or a cyanimine group, **K,** following Scheme 4. For the cyanoimine analog, the exchange of the oxygen occurred via condensation followed by the Michael addition of methanol to the cyanamine. The NBS acts as an oxidant and removes the hydrogen of the intermediate. For the thioketone analog,[16] Lawesson's reagent was used. Lawesson's reagent is a thionating agent specific to ketones. This exchange is facilitated by a cycloreversion step that resembles a portion of the Wittig reaction mechanism.[17] Based on various spectral data, the analogs were successfully synthesized. These analogs are beneficial in probing the active site for the importance of sterics and electronegativity.



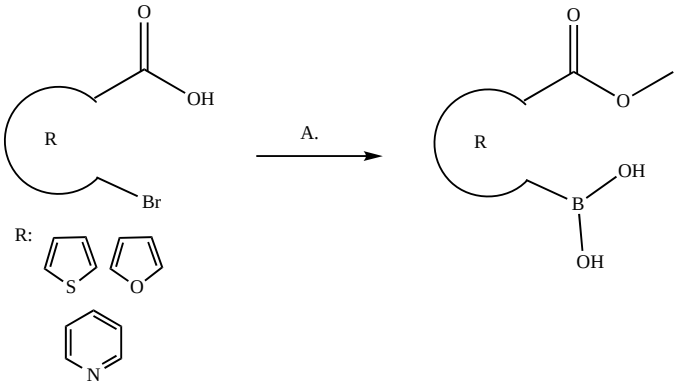
Scheme 4: Synthesis scheme for ketone analogs

A. MeOH, *t*-BuOK, H_2NCN, r.t.
B. Tolune, Lawesson's reagent, inert, 110 °C
X = NCN, S

3.4. Optimization of Heterocycle Synthesis

Although all the synthetic methods to make the protected and boronated heterocycles were successful in making the desired product, the yields were extremely low, requiring optimization. Initially, the carboxylic acid heterocycles were subjected to a Williamson ether synthetic scheme. Spectral data revealed that after eighteen hours, only a small fraction

of the starting material was methylated. To overcome this, the acid was first treated with thionyl chloride to form the highly reactive acid chloride which was then treated with methanol to produce the ester. Once the acid was protected, the crude reaction mixture was then subjected to a boronation reaction to produce the desired boronic acids. In order to increase the yields, this reaction was ran at room temperature overnight rather than for four hours in an ice bath, as was initially done. The optimized synthetic scheme is shown below in Scheme 5.



Scheme 5: Synthesis of boronic acid heterocycles

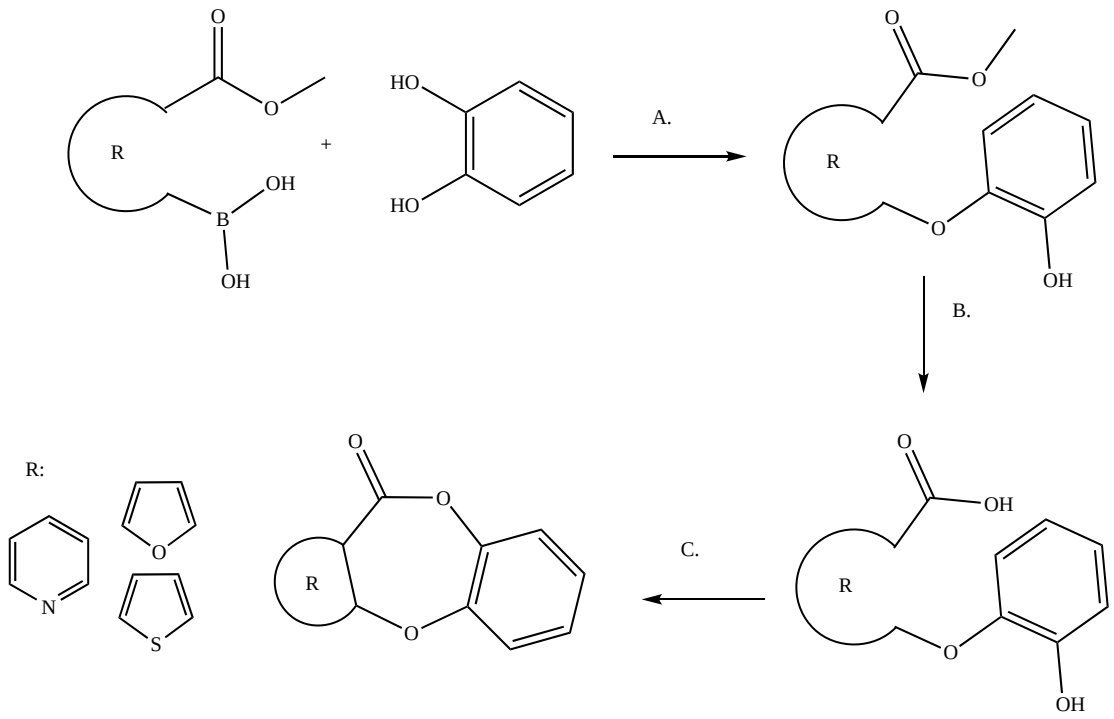
A. 1) Thionyl chloride, MeOH, THF
2) Mg, LiCl $(CH_3O_3)B$

3.5. Optimization of Copper Acetate Removal

One of the most confounding aspects of the core synthesis was the initial inability to adequately remove the copper acetate from the crude product in Step 1 of Scheme 2. Initially when using smaller scales, a small Celite plug and filtration was adequate in removing enough $Cu(OAc)_2$ to separate the product via column chromatography. Upon scaling up the reactions, the Celite plug proved inadequate for removing the copper acetate. Initially, chelators were used to try to complex the copper after filtration via a fritted funnel. Although the chelators were successful in removing some copper, there was still a great deal left in the crude mixture. It was then attempted to extract out the copper acetate using CH_2Cl_2 and water. This too proved to be insufficient. Finally, the crude reaction was filtered using a Celite plug via a fritted funnel and then stirred with EDTA and saturated aqueous $NaHCO_3$ for 20 minutes. Following the complexing with the EDTA, the EDTA was removed via extraction from the desired product using ethyl acetate and water. This was successful in removing all visible copper from the crude and enabled separation of the desired product using column chromatography. Although this is a successful method for removing the copper, this may have contributed to the extremely low yields of the A ring analogs.

3.6. A Ring Analog Synthesis

When attempting to make the A ring analogs, it proved difficult to reproduce similar yields to those seen in the unmodified core and the ether analogs. This could be due to the new method of copper removal or the difference in reactivity of the heterocyclic boronic acid derivatives relative to the unmodified boronic acid. The electron withdrawing capacities of the thiophene and the furan rings could be making it more difficult for the boronic acid to leave or for the deprotonated alcohol to attack. For the unmodified boronic acid, the benzene ring acts as an electron donator. Moreover, the heterocyclic boronic acid was made in the laboratory and not completely pure before using it in Scheme 6 whereas the unmodified boronic acid is bought from a chemical company and is pure. This could affect the yields of the desired products as other reaction pathways could be occurring and giving additional, unwanted byproducts.



Scheme 6: Ring A analog synthesis scheme

A. $Cu(OAc)_2$, pyridine, CH_2Cl_2
B. 2 M NaOH, 0.1 M CH_3OH
C. 20 mM THF. Thionyl chloride, pyridine

3.7. Antibacterial Assay Results

The unmodified core structure, **C**, and the analogs were tested for antibacterial activity against Gram-positive and Gram-negative bacteria using an antibacterial assay. The unmodified core structure showed antibiotic properties against Gram-positive and Gram-negative bacteria. The cyanoimine also showed activity against both Gram-positive bacteria. The S-curve for the cyanoimine analog against *Staphylococcus aureus* is depicted in Figure 8, and Figure 9 depicts the S-curve for the unmodified core against *Escherichia coli*. From the absorbance data, the IC_{50} of compound **K,** the cyanoimine analog, against *S. aureus* was 24.1 μg/mL. Several of the analogs showed visual activity against Gram-positive bacteria, but the coloration of the compound confounded the ability of the plate reader to see the activity. These compounds with visual activity were noted above, and no further quantitative analysis was explored.

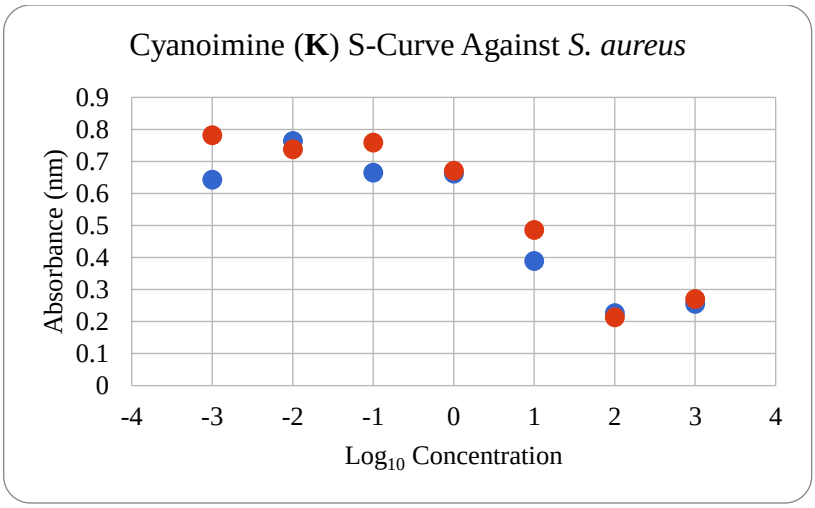


Figure 8: S-Curve of Cyanoimine Against *S. Aureus*

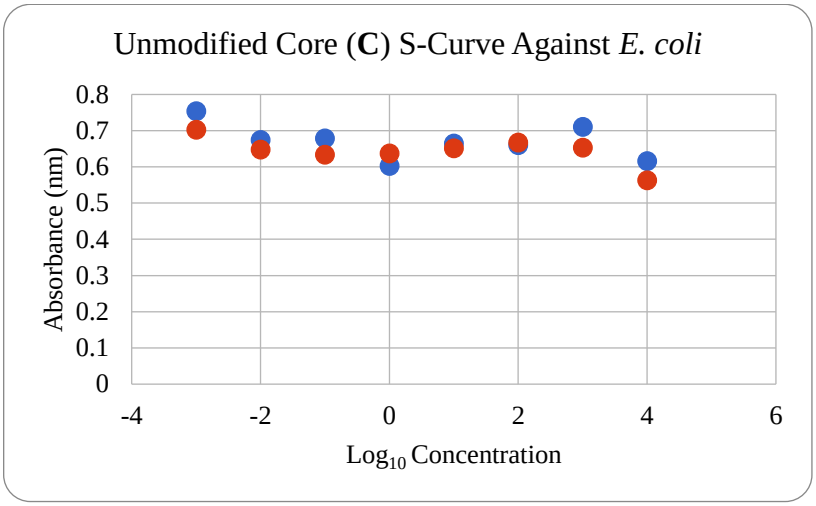


Figure 9: S-Curve of Unmodified Core Against *E. Coli*

With the compounds quantitatively analyzed, the color of the analogs confounded the data as it inhibited some of the absorbance from the highest concentrations. In order to accommodate for this, the confounding absorbance values were left out of the calculations and S-curve graphs. At the moment, there is not a method available that can overcome this problem.

4. Conclusion

This data illuminates which portions of the depsidone core are vital to its activity. It appears that the oxygen ether linkage is vital to its activity as neither the thioether nor the amine showed as potent activity against either type of bacteria. Additionally, the core also showed activity against Gram-negative bacteria, which has not yet been reported for this class of natural products. It can be concluded then that the higher electronegativity of oxygen relative to nitrogen or sulfur may be vital for the binding of the molecule to the active site. A way to further test this hypothesis may be to add a fluorine to this point of the molecule and observe how activity changes. Beyond electronegativity, oxygen acts a hydrogen bond acceptor. Nitrogen is a hydrogen bond acceptor, and sulfur does not participate in hydrogen bonding. Although it is difficult to draw conclusions on the bonds existing between the molecule and the

area of activity within the cell, it is possible that this hydrogen bond capacity of oxygen is a vital interaction. Until more data is collected on the mechanism of action of depsidones, no final conclusions can be drawn about any interactions between the molecule and the cell.

The cyanominine has a larger steric hindrance than the unmodified ketone. Since activity was observed for this analog, it can be hypothesized that the larger analog fills more space within the active site, promoting better binding. Although the thioester also showed visual activity against *Staphylococcus aureus*, the activity was not as strong as the cyanoimine. This further supports the hypothesis that steric effects help increase activity against Gram-positive bacteria in this position, and electronegativity and hydrogen bonding capacity are less critical. Testing analogs with tertiary amines or bulky hydrocarbon groups can help elucidate further the extent to which steric effect and hydrophobic versus electronic effects affect activity. It is important to note that none of the ketone analogs showed any activity against Gram-negative bacteria. This supports that less bulky substituents in this position promote activity against Gram-negative bacteraia.

Although the results of the study are still preliminary, conclusions can be drawn from the available data. As the electronegativity of the ether decreases, the antibacterial potency of the molecule also decreases or disappears completely. As the molecule is made bulkier at the ketone position the activity of the molecule increases for Gram-positive bacteria. The unmodified core showed activity against Gram-negative bacteria, which has not yet been reposted. This data aids in the elucidation of the structure of the active site and provides meaningful insights into ways to improve antibiotic potency of the depsidone core.

5. Acknowledgements:

The author wishes to express her appreciation to the UNC Asheville Chemistry department for facilitating an environment which promotes undergraduate research and their funding. The author would also like to acknowledge the North Carolina Biotechnology Center 2015-BRG-1201 for their funding. Thanks to the faculty within the chemistry department for their guidance, advice, and support throughout the project.

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