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Synthesis and Antibiotic Assessment of Pestalone Derived Aryl and C9 Analogs

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Abstract

Pestalone (1) is a natural product first isolated by W. Fenical et al. in 2001 from a cofermentation of a marine fungus and antibiotic-resistant marine bacterium. Pestalone was reported to have highly potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MIC = 37 ng/mL) and vancomycin-resistant *Enterococcus faecium* bacteria (MIC = 78 ng/mL). Consequently, this made pestalone a promising, new antibiotic compound. Due to its challenging chemical structure, total synthesis of 1 has only been achieved by Iijima et al. and Slavov et al. Unfortunately, the latter group reported multiple difficulties during and after total synthesis which include the facile intramolecular cyclization between the C9 aldehyde and bridging ketone forming a lactone, rendering it inactive and discrepancies in the degree of antibacterial activity. This work aims to synthesize C9 pestalone analogs incapable of undergoing the inactivating intramolecular cyclization by replacing the aldehyde with a range of electronic and steric functional groups. To date, eleven analogs were synthesized using a two-step synthesis involving first a Grignard addition of bromobenzene to a substituted phthalic anhydride (30-99% yield), and then modifying the produced carboxylic acid through esterification (47-76% yield) or amidation (7-50% yield). These analogs were then subjected to a broth microdilution minimum inhibitory concentration assay against *Staphylococcus aureus*, and it was found that only the analogs with the carboxylic acid showed activity. Continuing efforts are being made to access more analogs by synthesizing other substituted phthalic anhydrides.

1. Introduction

Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals and the community. The ability of bacteria to resist the effects of an antibiotic occurs when the bacteria have a random mutation that allows them to be naturally resistant to the antibiotic. This resistance is then passed on, leading to a proliferation of the resistant bacteria compared to the non-resistant. Currently, antibiotic resistance is viewed as one of the world's most pressing public health problems due to its ability to cause illnesses that were once treatable, to become dangerous infections that prolong suffering for children and adults and can ultimately lead to death. Dating back to the introduction of benzylpenicillins and sulfa drugs, it has been observed that when given enough time, bacteria will develop resistance to any antibiotic used in clinical practices.[1]

The challenge posed by resistance among Gram-positive bacteria is epitomized by methicillin-resistant *Staphylococcus aureus* (MRSA). *Staphylococcus aureus* is considered to be one of the prominent, medically important bacterial pathogens. MRSA infections are often fatal in nature, with an average of 20,000 deaths per year in the U.S alone, and are associated to resistance of several beta-lactam antibiotics. Life-threatening infections, which were limited to being only found in hospitals, are now spreading into communities worldwide. Conventional anti-MRSA antibiotics such as vancomycin, linezolid, and teicoplanin are currently in clinical use however, development of resistance to many of these drugs has been identified. Conversely, promising approaches to combating multidrug resistant bacteria are emerging; some include discovering new antibiotics as well as new classes of antibiotics,[2]

synthetic tailoring of current antibiotics by leaving the core intact but modifying the periphery chemical groups,[3] the use of nanoparticle green chemistry,[4] and searching underexplored ecological niches for natural products.[5]

Pestalone, a chlorinated, highly functionalized benzophenone, is a natural product that was originally isolated in 2001 by W. Fenical and co-workers from a cofermentation of a marine fungus, of genus *Pestalotia*, and antibiotic-resistant marine bacterium (Figure 1).[6]

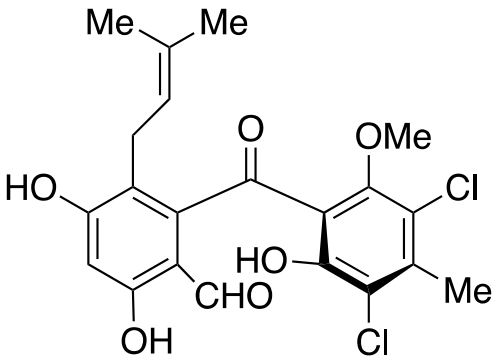


Figure 1. The marine natural product pestalone.[6]

Pestalone (1) was reported to have highly potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MIC = 37 ng/mL) and vancomycin-resistant *Enterococcus faecium* bacteria (MIC = 78 ng/mL), as well as acute toxicity due to the benzophenone backbone.[7, 8] Consequently, this made 1 a promising, new antibiotic compound. Due to its challenging chemical structure, total synthesis of 1 has only been achieved by Iijima et al.[8] and Slavov et al.[9] Unfortunately, the latter group reported multiple difficulties during and after total synthesis which include the facile intramolecular cyclization between the C9 aldehyde and bridging ketone forming a lactone (Figure 2), rendering it inactive and discrepancies in the degree of antibacterial activity.[9] Owing to its biological potential, limited availability from natural sources due to its extremely selective cofermentation, and challenging chemical structure 1 urges research aimed at its chemical synthesis.

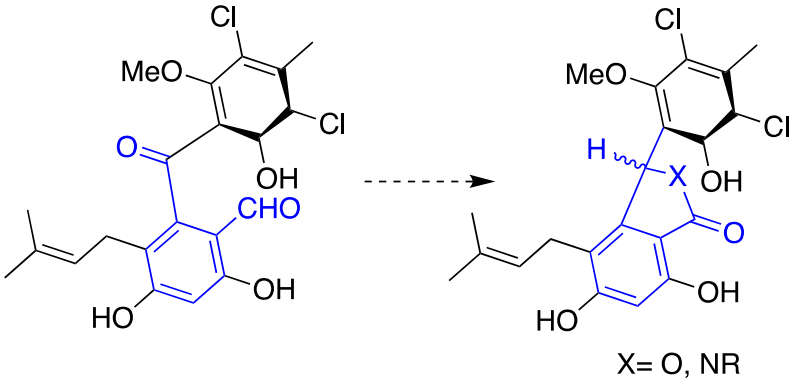


Figure 2. Intramolecular cyclization product.[9]

The difficulty of the total synthesis of 1 can be attributed to low yielding reactions. For this reason, research involving the total synthesis of 1 and analogs needs to continue pushing forward. Synthesizing and testing the antibacterial activity of 1 and a wide range of analogs will be important in understanding the molecular mechanism of action of 1 as an antibiotic compound, improve the field of medicinal chemistry and exist as a possible solution to a growing issue worldwide.

As previously mentioned, the total synthesis of 1 and structurally related compounds have been conducted by only a few research groups due to the challenging synthesis of 1. Slavov et al. was the first group to perform synthetic studies on the highly substituted antibiotic, 1, in 2003. Unfortunately, Slavov et al. were only able to produce deformyl-

derivatives, due to the difficulty of the final formylation, leading to a series of n-1 analogs.[9] It was not until 2004 that the first total synthesis of 1 was accomplished by Iijima et al. (Figure 3).[8]

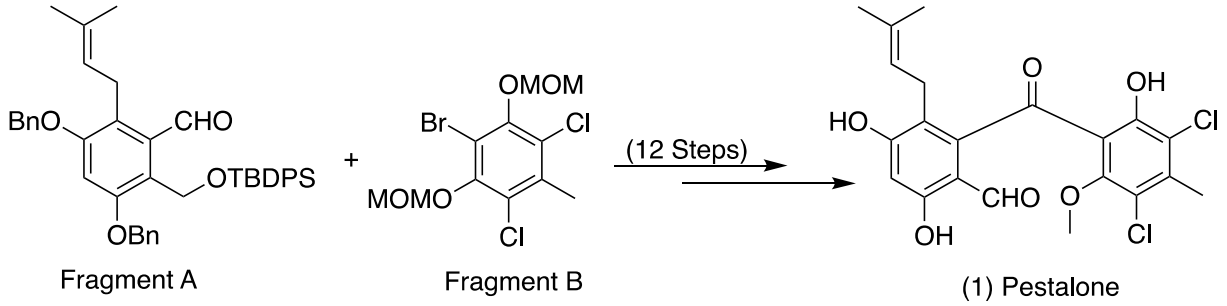


Figure 3. Forward synthesis of pestalone by Iijima et al. [8]

Shortly thereafter Slavov et al. was also able to accomplish a total synthesis of 1, which was confirmed by X-ray crystal structure analysis.[9] Iijima's synthetic scheme used 12 steps while Slavov and his team reported their synthetic scheme of 1 being highly efficient and practicable, only requiring 10 steps (Figure 4).[9] It is also important to note that while developing their scheme for total synthesis, Slavov et al. also reported, under certain conditions, 1's intramolecular cyclization created multiple pestalone-type compounds including a structure similar to a racemic natural product under neutral and slightly basic conditions.[9] Using the unique reactivity, they also developed a short and efficient synthesis of some structural analogs.

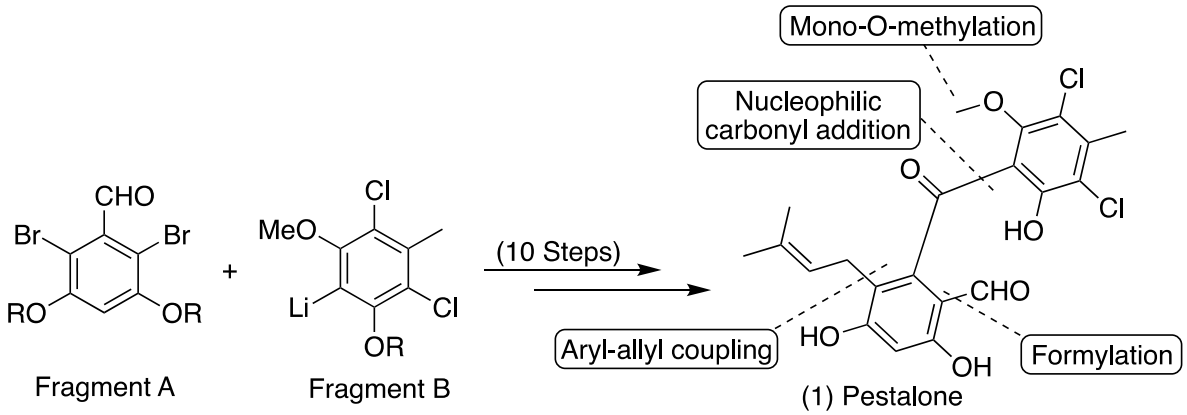


Figure 4. Retrosynthesis of pestalone by Slavov et al. [9]

Following the success of synthesizing 1 and the analogs, Slavov et al. used the opportunity to further explore the antibacterial potency of them. They utilized a standardized broth microdilution method to determine the activity against three different clinically relevant *S. aureus* strains. The reported results showed pestalone, pestalachloride A, and other analogs having a significant biological effect against S. *aureus* but only at high concentrations (MIC=10-25 µg/mL).[10] Remarkably, no further biological experiments have been reported since, which makes the research ideal for other laboratories to pick up and continue.

Prior work in the Wolfe Research Group has been done on synthesizing pestalone's carbon backbone, developing a microdilution minimum inhibitory concentration bacterial assay, and synthesizing and testing pestalone analogs. The research resulted in the development of a five-step synthetic pathway via Grignard and lithiate chemistry, oxidation, and carbonyl protection to synthesize the backbone. Afterwards, by way of Grignard reactions using substituted benzaldehydes or phthalic anhydrides, eleven pestalone analogs were synthesized with yields ranging from 65-85%.[11]

The chemical synthesis and antibacterial evaluation of 1 and its analogs are still areas that are under-researched. More experimentation needs to be done in order to increase yields, prevent intramolecular cyclization and lower the minimum inhibitory concentrations. In turn, we aimed to develop a robust synthetic scheme to produce aryl and C9

analogs of the antibiotic pestalone replacing the aldehyde with electronic and steric analogs in order to reduce the undesired intramolecular cyclization reaction while maintaining or improving antibacterial activity.

2. Experimental

2.1. General

All reagents were purchased as either reagent grade or ultra-pure anhydrous and used without further purification. Reactions that were moisture and air sensitive were run in flame- or oven-dried glassware under argon gas. The progress of each reaction was observed using thin-layer chromatography. Purification of each compound was accomplished using liquid-liquid extraction and column chromatography. Column chromatography purification was carried out using silica gel. The compounds were characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy techniques (Varian Gemini 2000 with an Oxford Instruments 400M Hz superconducting magnet) using $CDCl_3$ or methanol-d_4, infrared (IR) spectroscopy (Nicolet iS10), and liquid chromatography-mass spectrometry (LC-MS) (Shimadzu LC-MS 2020 respectively). Chemical shift values (δ) were reported in ppm with tetramethylsilane (TMS) as the internal standard.

2.2. General procedure for the synthesis of the 2-Benzoylbenzoic acids (1a-1d)

Bromobenzene (1 eq) was added to a mixture of 0.9M tetrahydrofuran (THF), magnesium (3 eq), and a catalytic amount of iodine at 0 °C. The mixture was stirred for 30 minutes at 0 °C, the temperature was then adjusted to 23 °C and stirred for 10 more minutes. After the 10 minutes at 23 °C the Grignard mixture was added to a solution of phthalic anhydride (1 eq) in 1M THF at 0 °C, 23 °C, or reflux depending on the specific substrate. This reaction stirred in between 2 hours or overnight. The solution was diluted with ethyl acetate and then washed with saturated aqueous sodium bicarbonate ($NaHCO_3$) X2. The aqueous layers were combined and acidified to pH<4 with 1N hydrochloric acid (HCl). Subsequently, the acidic layers were washed with ethyl acetate X3. The combined organic solutions were dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated under reduced pressure to yield the respective 2-benzoylbenzoic acids **(1a-1d)**.

2.3. Synthesis for Fisher Esterification (2a-2d)

The products **1a-1d** from the Grignard synthesis were used as the starting material for the esterification synthesis. The 2-benzoylbenzoic acid was added to a solution of methanol (0.25 M, 1 eq) or ethanol (0.25 M, 1 eq) and sulfuric acid (5 M, 1 eq) then stirred at reflux overnight. The solution was neutralized with $NaHCO_3$ and then extracted the aqueous layer with ethyl acetate X3. The organic extract was separated, dried over Na_2SO_4, and concentrated under reduced pressure to give the respective 2-benzoylbenzoates **(2a-2d)**.

2.4. Synthesis for Amidation (3a-3d)

The products **1a-1d** from the Grignard synthesis were used as the starting material for the amidation synthesis. The 2-benzoylbenzoic acid was added to a solution of 4-dimethylaminopyridine (DMAP) (0.25 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (3 eq), N, N-diisopropylethylamine (DIPEA) (3 eq), and dichloromethane (DCM) (0.04 M). Subsequently, an amine (1.5 eq) was added to the mixture and allowed to stir at 23 °C overnight. The resulting solution was diluted with deionized water and then extracted the organic layer with DCM X3. The combined organic extract was washed with saturated, aqueous sodium chloride (NaCl) solution X2, dried over Na_2SO_4, and concentrated under reduced pressure to yield the respective 2-benzoylbenzamides **(3a-3d)**.

2.5. Synthesis of Phthalic Anhydride (4)

Compound **4** was prepared according to the literature procedure.[12] Magnesium chloride (0.00200 g, 0.021 mmol), phthalic acid, and di-tert-butyl dicarbonate (0.220 g, 1.00 mmol) were dissolved in THF (1 mL). The reaction mixture

was stirred and heated at 40 °C for 19 h. Product was evaporated under reduced pressure to give **phthalic anhydride (4)**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.97 (m, 2H), 7.86 (m, 2H).

2.6. Synthesis of Thiophene-2, 3-dicarboxylic anhydride (5)

Compound **5** was prepared as previously reported.[13] In an inert environment, 3-bromothiophene-2-carboxylic acid (0.500 g, 2.42 mmol) was added dropwise to a stirring solution of n-butyllithium (2.90 mL) in dry diethyl ether (14.5 mL) at -78 °C. After ten minutes from the last addition, gaseous CO_2 was fluxed into the solution. The reaction stirred for 30 minutes at -78 °C then warmed to room temperature. Subsequently, the reaction was quenched with deionized water and extracted with 10% NaOH X3. The combined aqueous layers were acidified with 6N HCl X3. The combined organic layers were dried and concentrated under reduced pressure to give crude **thiophene-2, 3-dicarboxylic acid**. The crude product was then dissolved in acetic anhydride (1 mL) and stirred overnight at 140 °C to yield **thiophene-2, 3-dicarboxylic anhydride (5)** as pale yellow crystals (23%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 8.43 (d, 1H), 7.58 (d, 1H).

2.7. Synthesis of 2, 3-Pyridinedicarboxylic Anhydride (6)

Compound **6** was prepared according to the literature procedure.[14] Quinolinic acid (1.00 g, 5.98 mmol) was dissolved in acetic anhydride (2.5 mL) and stirred at reflux overnight. The reaction was then concentrated under reduced pressure to give **2, 3-Pyridinedicarboxylic Anhydride (6)** as a white solid (0.927 g, 48.1%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 9.11 (d, 1H), 8.34 (d, 1H), 7.82 (m, 1H).

2.8. Synthesis of 1-allyl-2-bromobenzene (7)

Compound **7** was prepared according to the literature with slight modifications.[15] In an inert environment, 2-bromobenzyl bromide (0.500 g, 2.00 mmol) was added to a solution of 0.1M anhydrous THF (20 mL). Subsequently, vinyl magnesium bromide (1.2 eq) was added dropwise to the solution at -78 °C. The mixture was stirred and allowed to warm to room temperature for 24 h. The reaction mixture was heated to reflux for an additional 24 h. Subsequently, 0.5 more equivalents of vinyl magnesium bromide was added to the solution and stirred overnight. After completion, the reaction was quenched with ammonium chloride, diluted with DCM, and the combined organic layer was washed with saturated, aqueous NaCl X3. The organic extract was separated, dried, and concentrated under reduced pressure to yield **1-allyl-2-bromobenzene (7)** as a brown wax. The yield was undetermined. Characterization in progress.

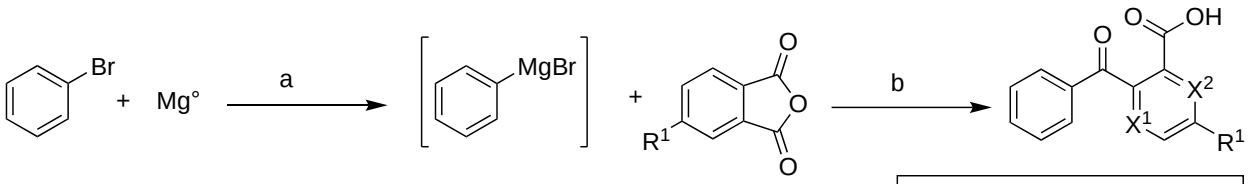
2.9. Standardized broth microdilution assay (OD_{590})

In a 96 well-plate, tryptic soy broth and varying concentrations, 1,000 μg/mL to 0.1 ng/mL, of each compound and the bacteria to be tested were added to corresponding wells. The plate was placed into an incubator at 37 °C overnight. Following the allotted time, the plate was removed and checked for bacterial growth. Each compound was tested against E. *coli* and S. *aureus*. Chloramphenicol was used as the control. The absorbance was measured using a Synergy HTX microplate reader at 590 nm (OD).

3. Results and Discussion

3.1. Chemistry

2-Benzoylbenzoic acids **1a-1d** (Scheme 1) were synthesized, via Grignard addition between bromobenzene and a substituted phthalide, to form the benzophenone carbon skeleton. The pure products were obtained in 30%-99% yield after acid-base extraction.

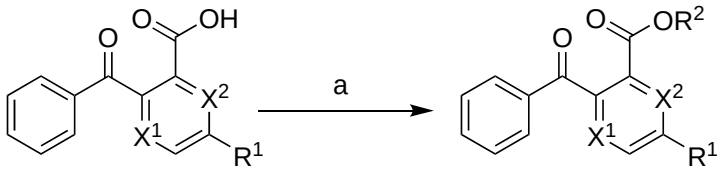


1a: R^1= H, X^1= CH, X^2= CH
1b: R^1= CH_3, X^1= CH, X^2= CH
1c: R^1= H, X^1= N, X^2= CH
1d: R^1= H, X^1= CH, X^2= N

Reagents and conditions: (a) I_2 (cat), THF, 0 °C to 23 °C, 24 h; (b) I_2 (cat), THF, 0 °C to 23 °C, 24 h, 30%-99%

Scheme 1. Synthetic route of compounds 1a-1d

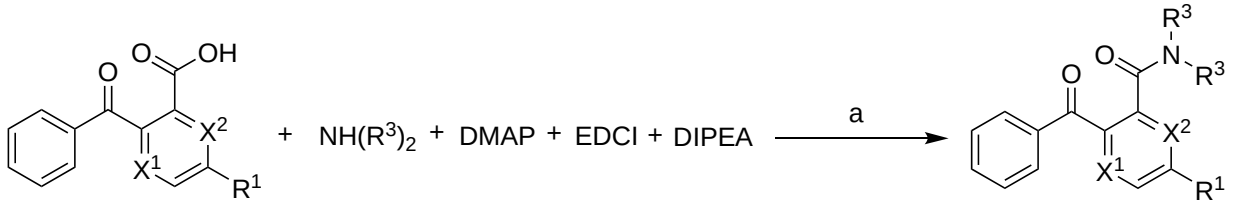
Once the requisite benzophenone derivative was synthesized, further modification of the carboxylic acid produced eight more analogs. Compounds **2a-2d** (Scheme 2) were prepared through Fisher esterification with 47%-76% yield and compounds **3a-3d** (Scheme 3) were prepared through traditional amidation with 7%-50% yield.



2a: R^1= H, R^2= CH_3, X^1= CH, X^2= CH
2b: R^1= H, R^2= CH_2CH_3, X^1= CH, X^2= CH
2c: R^1= CH_3, R^2= CH_3, X^1= N, X^2= CH
2d: R^1= H, R^2= CH_3, X^1= CH, X^2= N

Reagents and conditions: (a) R_2OH, H_2SO_4, Reflux, 2 h, 47%-76%

Scheme 2. Synthetic route of compounds 2a-2d



3a: R^1= H, R^3= CH_2CH_3, X^1= CH, X^2= CH
3b: R^1= H, R^3= H,Bn, X^1= CH, X^2= CH
3c: R^1= H, R^3= $CH_2CH_2CH_3$, X^1= CH, X^2= CH
3d: R^1= CH_3, R^3= CH_2CH_3, X^1= CH, X^2= CH

Reagents and conditions: (a) DCM, 23 °C, 24 h, 7%-50%

Scheme 3. Synthetic route of compounds 3a-3d

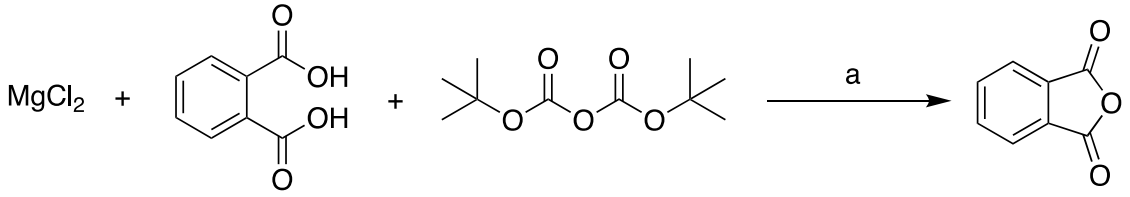
Eleven total analogs were synthesized. The analogs with electron donating groups produced lower yields. The progress of each reaction was observed using thin-layer chromatography. Purification of each compound was accomplished using liquid-liquid extraction and column chromatography, then characterized using ^{1}H NMR. Table 1 includes all the target compounds and their respective yields.

Table 1. Experimental Yields of Target Compounds 1a-1d, 2a-2d, and 3a-3d.

Compound	Yield	Compound	Yield	Compound	Yield
1a	30%	2a	76%	3a	31%
1b	99%	2b	47%	3b	24%
1c	37%	2c	59%	3c	50%
1d	37%	2d	65%	3d	7%

*Compounds with matching colors corresponds to having the same substituted B-ring

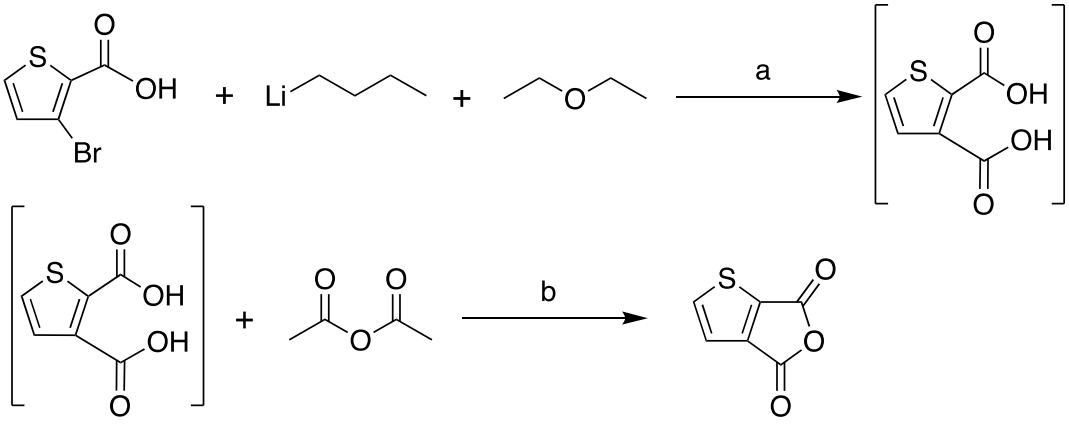
The cyclic anhydrides used in Scheme 1 were either bought commercially or made by way of organic base catalysis. Compound **4** was prepared by cyclization of the dicarboxylic acid using a Lewis acid catalyst prepared in situ from $MgCl_2$ and di-*tert*-butyl dicarbonate (Scheme 4).[12]



Reagents and conditions: (a) THF, 40 °C, 19 h

Scheme 4. Synthetic route of compound 4

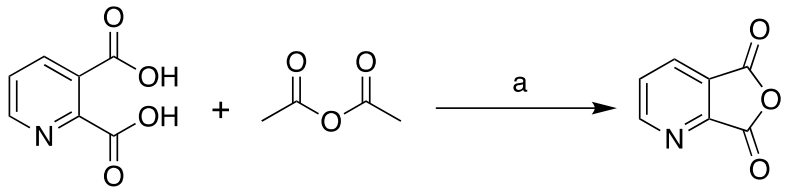
Compound **5** was prepared by doing a two-step synthesis, first synthesizing the dicarboxylic acid followed by the addition of the dehydrating agent, acetic anhydride (Scheme 5).[13]



Reagents and conditions: (a) Ar (g), CO_2 (g), -78 °C to 23 °C, 19 h; (b) 140 °C, 24 h

Scheme 5. Synthetic route of compound 5

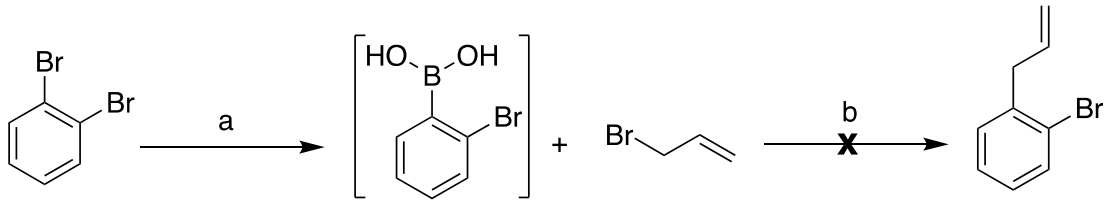
Compound **6** was obtained via a dehydration of the acid using acetic anhydride (Scheme 6).[14] Each cyclic anhydride was characterized using ^{1}H NMR spectroscopy techniques.



Reagents and conditions: (a) Reflux, 24 h, 48.1%

Scheme 6. Synthetic route of compound 6

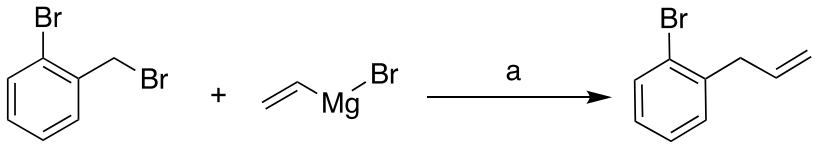
The synthesis of 1-allyl-2-bromobenzene (**7**) is currently in progress. Two different synthetic approaches were tried. The first attempt involved a Suzuki Coupling reaction using copper acetate catalyst (Scheme 7).



Reagents and conditions: (a) (i) Mg°, LiCl, THF, Ar (g), 23 °C, 24 h; (ii) $B(OCH_3)_3$, 0 °C, 3.5 h; (b) $Cu(OAc)_2$, pyridine, DCM, 23 °C, 24 h

Scheme 7. Synthetic route of compound 7

This approach proved to be difficult, which is most likely attributed to impurities in the crude (2-bromophenyl)boronic acid carried into the next step or the reaction not remaining dry during the second reaction step. The second attempt was a Grignard reaction with vinyl magnesium bromide (Scheme 8).[15] The success of the reaction is still being determined. If successful, this product can then be used in place of bromobenzene in Scheme 1.



Reagents and conditions: (a) Ar (g), THF, -78 °C to 23 °C, 24 h

Scheme 8. Synthetic route of compound 7

3.2. Antibacterial activities and structure-activity relationship (SAR)

Each analog was subjected to an in-house antibacterial assay against Gram-positive, *Staphylococcus aureus*, and Gram-negative, *Escherichia coli*, bacteria. The results from the assay can be used to determine their IC_{50} value (Figure 5). The analogs containing the carboxylic acid substituent exhibited the highest antibacterial activity.

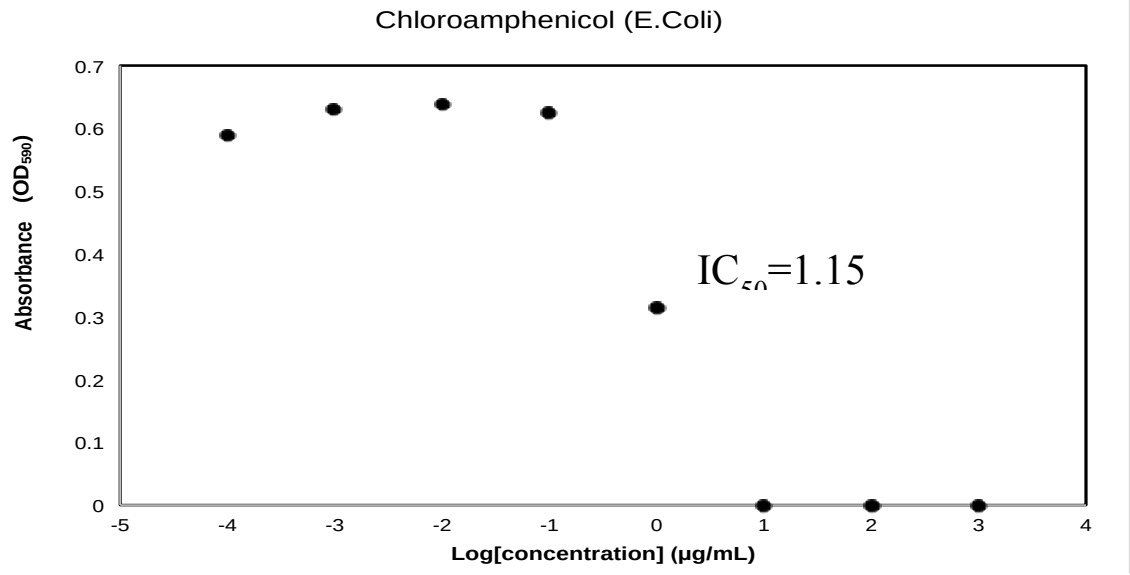


Figure 5. Graph of S curve for chloramphenicol from the E. *coli* assay with the IC_{50} value.

4. Conclusions and Future Directions

Overall, the analogs containing the carboxylic acid substituent showed the highest antibiotic activity when tested against an *E. coli* and *S. aureus* antibacterial assay. In the future, synthesis of new pestalone analogs and heterocyclic anhydrides will continue. A synthetic scheme for the addition of a methoxy onto the B-ring, and develop an A-ring like phthalic anhydride synthesis will be developed. Additionally, assay's will be run on existing analogs in order to determine their mechanism of action.

6. Acknowledgements

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