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Synthesis of Pestalone Analogs and Evaluation of Their Antibiotic Activity

Jordan Hartig
Chemistry
The University of North Carolina Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Amanda Wolfe

Abstract

Antibiotic resistance is one of the largest public health issues today. One way that antibiotic resistance can be prevented is through the introduction of novel antibiotics. Pestalone, a natural product derived from a deep-sea marine bacterium, has demonstrated activity against antibiotic resistant bacteria, most notably Methicillin-resistant Staphylococcus aureus. Pestalone is difficult to isolate from its natural source and prior synthesis have been low yielding, although it has been potent against bacteria. This work explores the structure activity relationship of a number of simplified Pestalone analogs that can be synthesized in 3-5 reactions with an overall yield for each synthesis of 65%-99%. Previously, at least twelve analogs of Pestalone has been synthesized with a series of reactions using substituted benzaldehydes, with Grignard reactions being an important step in each reaction. Recently, two additional analogs have been synthesized using this method and evaluated for their antibiotic activity. All synthesized analogs were tested in bacterial cell death assays against Gram-positive S. aureus and Gram-negative E. coli. Two of the twelve synthesized analogs demonstrated moderate activity against E. coli and one of the synthesized analogs showed activity against Staphylococcus aureus. Active analogs will be used in a synthesis that would add a carbon chain to the analogs.
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1. Introduction

Antibiotic resistance is one of the largest issues in modern medicine today. Recent statistics have shown that in the United States each year 2.8 million people contract an antibiotic resistant infection and of those 2.8 million people, more than 35,000 die from those infections.[1] There are a number of factors that can contribute to antibiotic resistance, including the over-prescription of antibiotics to a patient and a patient's failure to finish their entire course of antibiotics.[2] When antibiotic resistance occurs, it is usually caused by a few harmful bacteria in an environment developing a random genetic mutation that allows them to be resistant to antibiotic compounds. Once a bacteria-killing agent is administered, these few resistant bacteria are able to survive the attack and go on to reproduce, eventually causing a potentially devastating infection.[1,2] Antibiotics cause bacterial cell arrest or death by either penetrating the bacteria's cell wall to inhibit protein synthesis or DNA replication, which is used by the antibiotic Tetracycline[3], or by acting directly upon the cell wall, a technique used by Penicillin[4]. Moreover, antibiotics can also target other structures in bacteria such as DNA gyrase (fluoroquinolones)[5] and ATP Synthase (Bedaquiline)[6]. In order to continue to treat bacterial infections that are resistant to existing antibiotics, scientists must either discover new antibiotics through natural product isolation and de novo drug design or improve the potency of known antibiotics through synthetic derivation.[7]

One natural product that has been shown to have antibiotic activity is the compound Pestalone (Figure 1).

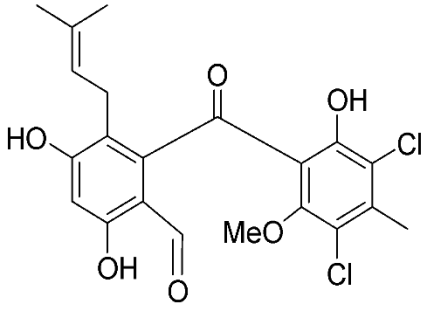


Figure 1. Pestalone

Pestalone was first isolated from the co-culture of a marine fungus *Pestalotia* and a unicellular marine bacterium (CNJ-328) by Mercedes Cuerto et. al. in the Bahamas Islands in 2001. Its structure was confirmed by single-crystal X-ray analysis. When tested against bacterial pathogens *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E.*

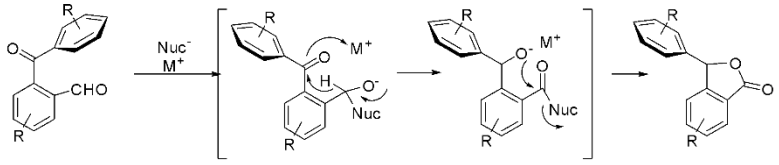
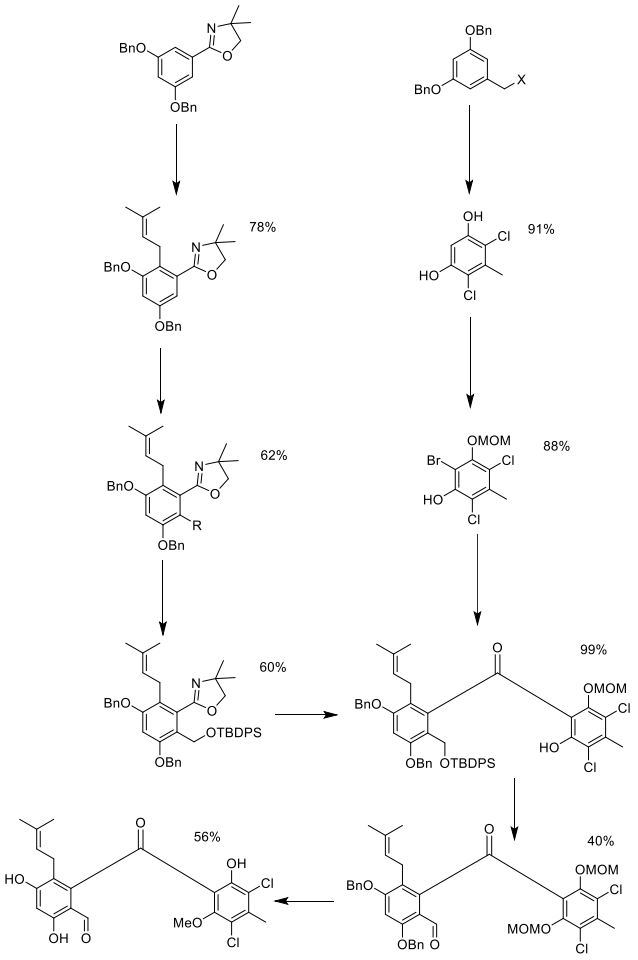


Figure 2. Spontaneous rearrangement of Pestalone to Pestalalactone

coli) (including methicillin resistant *S. aureus*), it was shown to have minimum inhibitory concentrations (MIC) of 37 ng/mL against *S. aureus*. Cueto also discovered that Pestalone has anti-cancer properties as well as activity against vancomycin-resistant *Enterococcus faecium* (VRE),[8] making it an excellent candidate in the fight against antibiotic resistance. However, despite these medicinal properties, natural product isolation of Pestalone is very difficult, and the synthesis of Pestalone is typically low-yielding, which reduces the amount of compound able to be produced and evaluated.[8,9] Additionally, despite the promising activity of Pestalone, it can undergo a spontaneous reaction under neutral and basic conditions that involves the rearrangement of the carbonyl group and alcohol group into a lactone ring (Figure 2), which converts Pestalone into Pestalalactone. This reaction is detrimental because once Pestalone is converted into Pestalalactone, it loses all antibiotic properties.[10,11] Therefore, the overall goal of this research is to synthesize analogs of the compound Pestalone that increase antibiotic activity for use against *S. aureus* and *E. coli* infections while also replacing the reactive aldehyde with similar functional groups that cannot undergo the undesired cyclization.

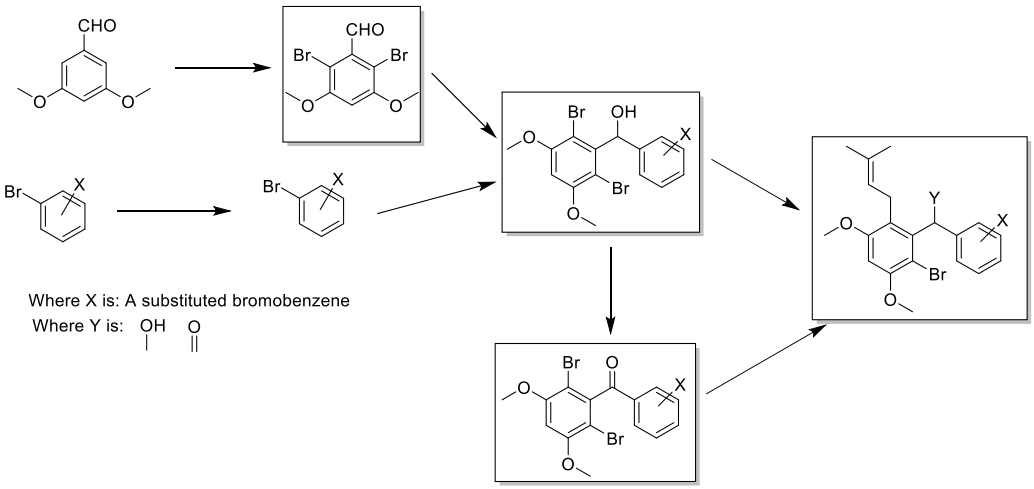
Previous research on Pestalone includes the initial discovery and isolation of Pestalone by Mercedes Cueto et. al. [8] and further research completed by Daisuke Iijima et. al.[9] This group reported the first total synthesis of Pestalone as well as the compound of SB87-Cl, which were both shown to have antimicrobial activity against MRSA and VRE. They completed the synthesis by using two substituted benzyl rings and combining them together using a Grignard reaction to form the bicyclic structure of Pestalone (Scheme 1). They also explored another method of using phthalic anhydride and a substituted benzyl ring in a Grignard reaction that lead to the bicyclic structure of Pestalone.[9] Further research was conducted by Nikolay Slavov et. al. This group probed the structure of Pestalone and discovered its facile conversion from Pestalone to Pestalalactone. This group used the synthesis method that involved the use of two substituted benzyl rings to complete a Grignard reaction that lead to the bicyclic structure of Pestalone. Their research ultimately revealed that after synthesis, Pestalone underwent a Cannizzaro-Tishchenko-type reaction that lead to the formation of a lactone. Slavov et. al. then confirmed that once the synthesized Pestalone undergoes the formation of a lactone ring, it loses its antibacterial properties, rendering it useless as an antibiotic.[10] To date, the major findings in the field of Pestalone synthesis detail a number of synthetic schemes that can be followed in order to successfully synthesize Pestalone or analogs of Pestalone. Other findings detail the issue of the lactone formation in Pestalone after completed synthesis and how the possibility of synthesizing analogs of Pestalone may avoid this.[11]

Previous work in the synthesis of Pestalone conducted by Dr. Wolfe's research laboratory was done by both Rhapsody Taylor and Madilyn Snyder. Both worked to synthesize analogs of Pestalone that are active against both *S. aureus* and *E. coli*. Rhapsody Taylor worked to synthesize analogs of Pestalone by following two synthetic pathways. One pathway probed the formation of the bicyclic structure of Pestalone by reacting a Phthalic Anhydride with a benzyl ring via Grignard reactions in order to form the frame used to attach different functional groups that would mimic the structure of Pestalone. The other synthetic pathway that she followed included the combination of two substituted benzyl rings containing a multitude of different functional groups in order to create the bicyclic structure of Pestalone with functional groups that can be substituted in order to further mimic the structure of Pestalone in the hopes of increasing the product's activity against bacteria.[11] Madelyn Snyder worked to synthesize analogs of Pestalone by using the synthesis scheme that involved the use of substituted phthalic anhydride and benzyl groups and then modifying the products of these syntheses by substitution of different functional groups.[12] Both researchers utilized cell death assays to analyze the effectiveness of their synthesized analogs and both found that the carboxylic acid analogs at the C9 position showed modest activity against *S. aureus*.[11,12]



Scheme 1. First total synthesis of Pestalone with the percent yield of each reaction, adapted from Iijima et. al.

The goal of this research was to develop a synthetic scheme to follow in order to synthesize a number of analogs that have the bicyclic structure of Pestalone (Scheme 2) primarily using basic reactions from literature that utilize the two benzyl group Grignard reactions. Once a number of analogs were synthesized, they were analyzed using a cell death assay that assessed their antibiotic properties. The analogs that demonstrated the most potent antimicrobial properties were further modified in an attempt to increase antibiotic properties.



Scheme 2. Proposed synthetic scheme for the synthesis of simplified analogs of Pestalone with proposed analogs highlighted

2. Materials and Methods:

2.1 General

Reagents and solvents were purchased as reagent-grade and used without further purification. All reactions were performed in flame-dried glassware under an Ar or N_2 atmosphere. Evaporation and concentration in vacuo was performed at 40 °C. TLC was conducted using precoated SiO_2 60 F254 glass plates from EMD with visualization by UV light (254 or 366 nm). NMR (^1H or ^{13}C) were recorded on an Oxford Varian-400 spectrophotometer at 298K. Residual solvent peaks were used as an internal reference. Coupling constants (*J*) (H,H) are given in Hz. Coupling patterns are designated as singlet (s), doublet (d), triplet (t), quadruplet (q), quintuplet (qu), multiplet (m) or broad singlet (br). IR spectra were recorded on a Thermo Scientific Nicolet iS10 FT-IR spectrophotometer and measured neat. Low-resolution mass spectral data were acquired on both a Shimadzu single quadrupole LCMS-2020 and a Shimadzu triple quadrupole LCMS-8040.[13,14]

2.2 3,5-Dimethoxybenzaldehyde Substitution General Procedure

Under an inert Ar atmosphere, 3,5 dimethoxybenzaldehyde was dissolved in 0.1M CH_3CN and combined with N-bromosuccinimide (2.3 eq) at 0 °C. The ice bath was removed and the reaction was allowed to warm to room temperature and stir until a white precipitate appeared (usually between 4 and 24 hours). The precipitate was collected via filtration with a fritted funnel after completion of the reaction. This precipitate was washed with CH_3CN at 0 °C and concentrated under reduced pressure. The product was then characterized.[13,14]

2.3 Bicyclic Structure General Grignard Procedure

At 0 °C, Mg^o (3 eq) and a catalytic amount of I_2 were combined in dry THF under an inert Ar atmosphere. Bromobenzene (1 eq) was added to the reaction and it was stirred at 0°C for 30 minutes. The ice bath was removed after 30 minutes and the reaction turned first yellow, then green. After stirring for 40 additional minutes, the Grignard reagent (1 eq) was added in 1 M dry THF at 0 °C via syringe. The reaction was stirred for 20 minutes at 0 °C, and then warmed to room temperature for an additional 30 minutes. The reaction was then poured into a separatory funnel and diluted with ethyl acetate and extracted with saturated aqueous $NaHCO_3$ three times. The organic layers were combined and dried over Na_2SO_4. The product was then concentrated under reduced pressure, purified via SiO_2 column chromatography, and characterized.[13,14]

2.4 Conversion from Alcohol to Carbonyl General Procedure

Under an inert Ar atmosphere, pyridinium chlorochromate (PCC) (2 eq) was partially dissolved in dichloromethane (DCM) (0.12 M) in an inert flask containing molecular sieves. In a separate inert flask, the product from Bicyclic Structure General Grignard Procedure was dissolved in THF (0.47 M) and added to the first flask via syringe. The reaction was allowed to stir at room temperature until complete as determined by TLC (~4-24 hours). The solution was then filtered via fritted funnel and the filter cake was washed with DCM. The filtrate was concentrated under reduced pressure and characterized.[13,14]

2.5 Chain Addition General Procedure

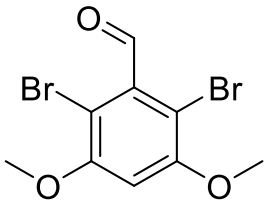
A solution of the Bicyclic Grignard Product (1 eq) was dissolved in THF (1 eq) in a flame dried flask under Ar and cooled to -78 °C. Then, Methyl lithium (1.3 eq) was added to the reaction via syringe. After stirring for 30 minutes, prenyl bromide (2 eq) was added and the reaction continued to stir for 3 hours at -78 °C. Once complete, excess reagent was quenched using a 1:1 mixture of brine and ammonia hydroxide. The reaction was then extracted with ethyl acetate (3X) and the organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure.[15]

2.6 Bacterial Assay General Procedure

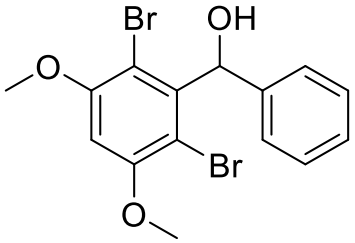
To achieve sterile conditions, both the workbench and gloves were washed with ethanol. Work completed under sterile conditions was completed under a flame produced by a propane torch. Lids for sterile containers were flamed before being replaced on the container.

Under sterile conditions, approximately 4 mL of full-strength tryptic soy broth (FSTSB) was transferred into conical tubes via Eppendorf pipette. To each test tube, a single colony of a chosen bacteria was added. At least one control test tube was present containing only FSTSB and no bacteria to ensure there was no contamination present. Bacterial colonies were grown in the prepared conical tubes by incubating while shaking the test tubes for 18 hours at 37 °C.

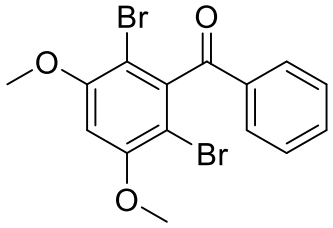
Once the bacteria were grown, assay plates were prepared under sterile conditions. Samples were diluted to 100 mg/mL in DMSO with a minimum final volume of 20 µL. The master plate was prepared by pipetting 9 µL of DMSO into each of the 96 wells on the 96 well plate except for the wells in the top row (row A). The top row of each plate contained either 10 µL of each compound being studied dissolved in DMSO, the antibiotic compound chloramphenicol diluted to 100 mg/mL as the positive control, or pure DMSO as the negative control. Tenfold dilution was achieved by taking 1 µL from the top row and mixing it into the well below it down each column. Test plates were filled with 89 µL of FSTSB per well, 10 µL of overnight culture, and 1 µL of each corresponding master plate well under sterile conditions. The plates were then incubated over night while shaking for 18 hours at 37 °C. Absorbance was read for each well via BioTek plate-reader at 590 nm using Standard *S. Aureus* Assay Protocol 590nm_blanks.prt and analyzed using Gen5 2.09 software data package.



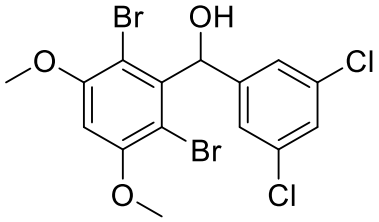
2,6 dibromo-3,5-dimethoxybenzaldehyde (1.1) 3,5 Dimethoxybenzaldehyde Substitution General Procedure. 3,5 dimethoxybenzaldehyde (6 g, 36 mmol), CH_3CN (270 mL, 0.13 M). N-bromo succinimide (15g, 83 mmol) was added, reaction was cooled to 0 °C and then allowed to warm to room temperature while stirring overnight. Filtration (fritted funnel, 200mL CH_3CN to wash) white precipitate. 98% yield. **H-NMR:** ($CDCl_3$-*d*, 400 MHz) δ10.36 (s, *J*=9.60, 1 H), 6.63 (s, *J*= 7.26, 1H), 3.393 (t, *J*=0.86, 6H) **C-NMR:** ($CDCl_3$-*d*) δ 191.0, 157.5, 157.5, 145.3, 110.6, 110.03, 110.03, 55.1, 55.1



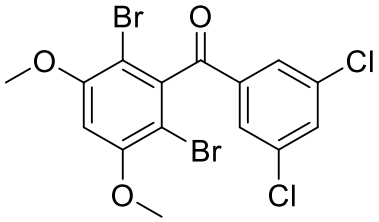
(2,6-dibromo-3,5-dimethoxyphenyl)(phenyl)methanol (1.2): Bicyclic Structure General Grignard Procedure. Under inert conditions, bromobenzene (1 g, 64 mmol), magnesium turnings (0.5g, 191 mmol), catalytic I_2, dry THF (7.0 mL, 0.9 M). Stirred at 0 °C for 30 minutes, ice bath was removed, reaction stirred at room temperature for 40 minutes, turning yellow then green. 2,6 dibromo-3,5-dimethoxybenzaldehyde (1g, 6 mmol) was added in dry THF (6.0 mL, 1.0M) at 0 °C via syringe and stirred for 20 minutes. Stirred at room temperature for 30 minutes. Column chromatography (500mL, SiO_2, 20% EtOAc/hexanes solvent system), 50% yield. **H-NMR:** ($CDCl_3$-*d*, 400 MHz) δ 7.38 (s, *J*=7.26, 1H), 7.27 (s, *J*=7.26, 2H), 7.18 (s, *J*=7.26, 1H), 6.43 (s, *J*=4.20, 1H), 6.32 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 3.93 (t, *J*=0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 158.5, 158.5, 151.6, 143.4, 129.2, 129.2, 128.2, 128.2, 126.2, 107.4, 107.4, 102.3, 69.2, 55.1, 55.1



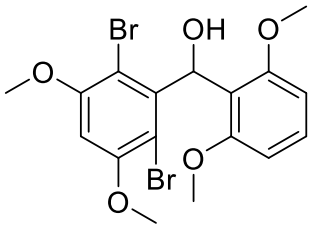
(2,6-dibromo-3,5-dimethoxyphenyl)(phenyl)methanone (1.3): Conversion from Alcohol to Carbonyl General Procedure. PCC (0.1 g, 0.5 mmol), DCM (2 mL, 0.1 M). In separate flask, (2,6-dibromo-3,5-dimethoxyphenyl)(phenyl)methanol (0.1g, 0.3 mol), THF (0.6 mL, 0.5 M), added to first flask via syringe. Reaction ran at room temperature for 4 hours. Filtered via fritted funnel and DCM to remove excess PCC, concentrated under reduced pressure. 76% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.75 (s, *J*=7.26, 2H), 7.61 (s, *J*=7.26, 1H), 7.51 (s, *J*=7.26, 2H), 6.54(s, *J*=7.26, 1H), 3.93 (t, *J*=0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 196.3, 156.7, 156.7, 146.4, 138.4, 130.3, 130.3, 128.4, 128.4, 110.7, 110.7, 108.5, 55.1, 55.1



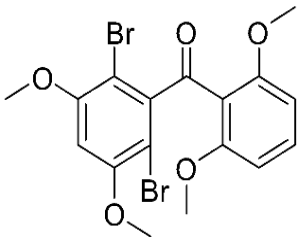
(2,6-dibromo-3,5-dimethoxyphenyl)(3,5-dichlorophenyl)methanol (1.4): Bicyclic Structure General Grignard Procedure. Under inert conditions, 1-Bromo-3,5-dichlorobenzene (1.8g, 8 mmol), magnesium turnings (0.6g, 24 mmol), catalytic I_2, dry THF (9mL, 0.9M). Stirred at 0 °C for 30 minutes, ice bath was removed, reaction stirred at room temperature for 40 minutes, turning yellow then green. 2,6 dibromo-3,5-dimethoxybenzaldehyde (3g, 8 mmol) was added in dry THF (8mL, 1.0M) at 0°C via syringe and stirred for 20 minutes. Stirred at room temperature for 30 minutes. Column chromatography (500mL, SiO_2, 10% EtOAc/hexanes solvent system), 38% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.58 (s, *J*=7.26, 1H), 7.34 (s, *J*=7.26, 2H), 6.43 (s, *J*=4.20, 1H), 6.32 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 3.93 (t,0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 158.5, 158.5, 151.6, 143.0, 136.2, 136.2, 126.7, 126.1, 126.1, 107.4, 107.4, 102.3, 68.2, 55.1



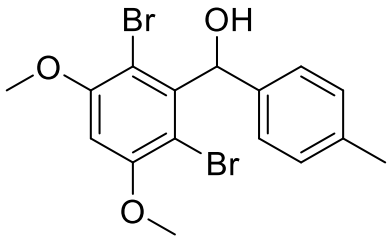
(2,6-dibromo-3,5-dimethoxyphenyl)(3,5-dichlorophenyl)methanone (1.5): Conversion from Alcohol to Carbonyl General Procedure. PCC (1g, 6 mmol), DCM (26 mL, 0.1 M). In separate flask, (2,6-dibromo-3,5-dimethoxyphenyl)(3,5-dichlorophenyl)methanol (1 g, 3 mmol), THF (7 mL, 0.5 M), added to first flask via syringe. Reaction ran at room temperature for 24 hours. Filtered via fritted funnel and DCM to remove excess PCC, concentrated under reduced pressure. 61% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.80 (s, *J*=7.26, 1H), 7.57 (s, *J*=7.26, 2H), 6.54 (s, *J*=7.26, 1H), 3.93 (t, *J*=0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 196.3, 156.7, 156.7, 146.4, 142.5, 132.9, 135.4, 135.4, 129.5, 129.5, 110.7, 110.7, 108.5, 55.1, 55.1



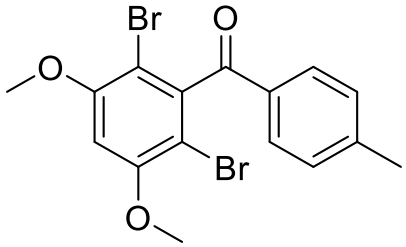
(2,6-dibromo-3,5-dimethoxyphenyl)(2,6-dimethoxyphenyl)methanol (1.6): Bicyclic Structure General Grignard Procedure. Under inert conditions, 2-bromo1,3-dimethoxybenzene (2 g, 9 mmol), magnesium turnings (0.7 g, 27 mmol), catalytic I_2, dry THF (97 mL, 0.9 M). Stirred at 0 °C for 30 minutes, ice bath was removed, reaction stirred at room temperature for 40 minutes, turning yellow then green. 2,6 dibromo-3,5-dimethoxybenzaldehyde (3 g, 9 mmol) was added in dry THF (108 mL, 1 M) at 0 °C via syringe and stirred for 20 minutes. Stirred at room temperature for 30 minutes. Column chromatography (500mL, SiO_2, 20% EtOAc/hexanes solvent system), 95% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.36 (s, *J*=7.26, 1H), 6.89(s, *J*=4.20, 1H), 6.61 (s, *J*=7.26, 2H), 6.32 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 3.93 (t, *J*=0.86, 2H), 3.72 (t, *J*=0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 158.8, 158.8, 158.5, 158.5, 151.6, 128.2, 124.7, 117.7, 117.7, 107.4, 107.4, 102.3, 57.4, 55.1, 56.1, 55.1, 56.1



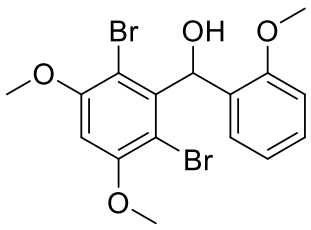
(2,6-dibromo-3,5-dimethoxyphenyl)(2,6-dimethoxyphenyl)methanone (1.7): Conversion from Alcohol to Carbonyl General Procedure. PCC (0.3 g, 1 mmol), DCM (11 mL, 0.1 M). In separate flask, (2,6-dibromo-3,5-dimethoxyphenyl)(2,6-dimethoxtphenyl)methanol (0.3 g, 0.7 mmol), THF (1 mL, 0.5 M), added to first flask via syringe. Reaction ran at room temperature for 24 hours. Filtered via fritted funnel and DCM to remove excess PCC, concentrated under reduced pressure. 4.61% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.58 (s, *J*=7.26, 1H), 6.85 (s, *J*=7.26, 2H), 6.54 (s, *J*=7.26, 1H), 3.93 (t, *J*=0.86, 2H), 3.90 (t, *J*=0.86, 2H) **C-NMR:** ($CDCl_3$-*d*) δ 196.3, 162.5, 162.5, 156.7, 156.7, 146.4, 134.4, 110.7, 110.7, 108.5, 106.3, 106.3, 105.1, 55.1, 55.8, 55.1, 55.8



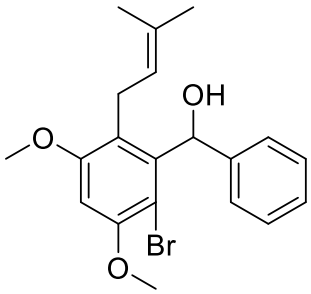
(2,6-dibromo-3,5-dimethoxyphenyl)(*p*-tolyl)methanol (1.8): Bicyclic Structure General Grignard Procedure. Under inert conditions, 4-bromotoluene (1.055g, 6.17 mmol), magnesium turnings (0.5 g, 19 mmol), catalytic I_2, dry THF (7 mL, 0.9 M). Stirred at 0ºC for 30 minutes, ice bath was removed, reaction stirred at room temperature for 40 minutes, turning yellow then green. 2,6 dibromo-3,5-dimethoxybenzaldehyde (2 g, 6 mmol) was added in dry THF (6 mL, 1 M) at 0 °C via syringe and stirred for 20 minutes. Stirred at room temperature for 30 minutes. Column chromatography (500mL, SiO_2, 10% EtOAc/hexanes solvent system), 75% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.23 (s, *J*=7.26, 2H), 7.09 (s, *J*=7.26, 2H), 6.43 (s, *J*=4.20, 1H), 6.32 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 3.93 (t, *J*=0.86, 2H), 2.19 (t, *J*=0.86, 1H) **C-NMR:** ($CDCl_3$-*d*) δ 158.5, 158.5, 158.5, 151.6, 140.4, 135.9, 129.5, 129.5, 129.5,129.5, 127.1, 127.1, 127.1, 107.4, 107.4, 102.3, 69.2, 55.1, 55.1, 21.3



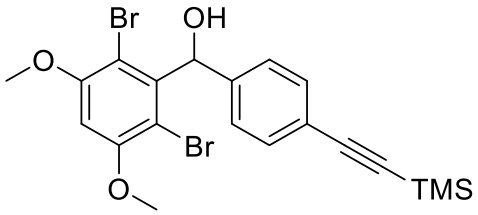
(2,6-dibromo-3,5-dimethoxyphenyl)(*p*-tolyl)methanone (1.9): Conversion from Alcohol to Carbonyl General Procedure. PCC (1 g, 5 mmol), DCM (40 mL, 0.1 M). In separate flask, (2,6-dibromo-3,5-dimethoxyphenyl)(*p*-tolyl)methanol (1 g, 2 mmol), THF (15 mL, 0.5 M), added to first flask via syringe. Reaction ran at room temperature for 4 hours. Filtered via fritted funnel and DCM to remove excess PCC, concentrated under reduced pressure. 69% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.66 (s, *J*=7.26, 2H), 7.28 (s, *J*=7.26, 2H), 6.54 (s, *J*=7.26, 1H), 3.93 (t, *J*=0.86, 2H), 2.41 (t, *J*=0.86, 1H) **C-NMR:** ($CDCl_3$-*d*) δ 196.3, 156.7, 156.7, 146.4, 142.1, 135.4, 130.2, 130.2, 128.7, 128.7, 110.7, 110.7, 108.5, 55.1, 55.1, 21.3,



2,6-dibromo-3,5-dimethoxyphenyl)(2-methoxyphenyl)methanol (1.10): Bicyclic Structure General Grignard Procedure. Under inert conditions, 1-bromo-2-methoxybenzene (2 g, 9 mmol), magnesium turnings (1 g, 28 mmol), catalytic I_2, dry THF (15 mL, 0.9 M). Stirred at 0 °C for 30 minutes, ice bath was removed, reaction stirred at room temperature for 40 minutes, turning yellow then green. 2,6 dibromo-3,5-dimethoxybenzaldehyde (3 g, 9 mmol) was added in dry THF (14 mL, 1 M) at 0 °C via syringe and stirred for 20 minutes. Stirred at room temperature for 30 minutes. Column chromatography (500mL, SiO_2, 20% EtOAc/hexanes solvent system), 1% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.28 (s, *J*=7.26, 1H), 7.01 (s, *J*=7.26, 1H) 6.93 (s, *J*=7.26, 2H), 6.89 (s, *J*=4.20, 1H), 6.32 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 3.93 (t, *J*=0.86, 2H), 3.72 (t, *J*=0.86, 1H) **C-NMR:** ($CDCl_3$-*d*) δ 158.5, 158.5, 154.7, 151.6, 134.9, 129.2, 127.2, 125.2, 112.8, 107.4, 107.4, 102.3, 63.3, 55.1, 55.1, 56.1

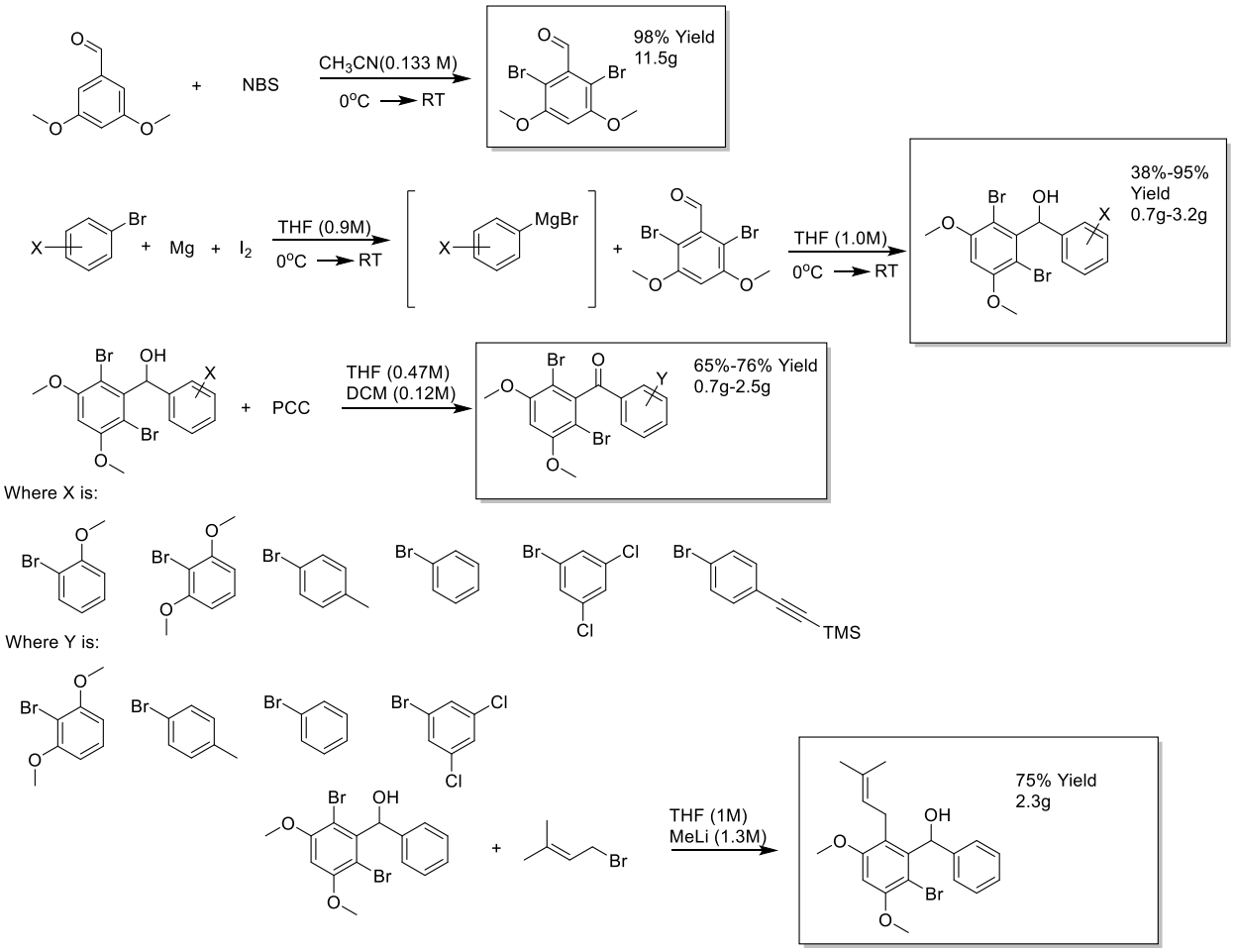


(2-bromo-3,5-dimethoxy-6-(3-methylbut-2-en-1-yl)phenyl)(2,6-dimethoxyphenyl)methanol (1.11): Chain Addition General Procedure. Under inert conditions, (2,6-dibromo-3,5-dimethoxyphenyl)(phenyl)methanol (2 g, 5 mmol), dry THF(61 mL, 5 M). Cooled to -78 °C and stirred. Added Methyl Lithium (4 mL, 7 mmol) via syringe, stirred for 30 minutes. Added prenyl bromide (1 mL, 11 mmol) and stirred at -78 °C for 3 hours. Quenched excess reagent with brine and NH_3, extracted with EtOAc, dried with $MgSO_4$. Column chromatography (500mL, SiO_2, 10% EtOAc/hexanes solvent system), 75% yield. **H-NMR:**($CDCl_3$-*d*, 400 MHz) δ 7.36 (s, *J*=7.26, 2H), 7.27 (s, *J*=7.26, 2H), 7.18 (s, *J*=7.26, 1H), 6.43 (s, *J*=7.26, 1H), 6.16 (s, *J*=1.50, 1H), 5.75 (s, *J*=5.25, 1H) 5.73 (s, *J*=4.20, 1H), 3.93 (t, *J*=0.86, 1H), 3.72 (t, *J*=0.86, 1H), 3.42 (d, *J*=1.37, 1H), 1.82 (t, *J*=0.86, 1H), 1.70 (t, *J*=0.86, 1H), **C-NMR:** ($CDCl_3$-*d*) δ 159.1, 158.8, 158.8, 156.5, 149.0, 128.2, 125.7, 124.7, 117.7, 117.7, 105.1, 94.9, 59.1, 55.1, 56.1, 56.1, 56.1, 24.4, 131.8, 123.1, 24.6, 18.6



(2,6-dibromo-3,5-dimethoxyphenyl)(4-((trimethylsilyl)ethynyl)phenyl)methanol (1.12): Bicyclic Structure General Grignard Procedure with some modification. Under inert conditions, ((4-bromophenyl)ethynyl)trimethylsilane (1 g, 0.4 mmol) magnesium turnings (0.3 g, 1 mmol, metallic coating stripped with a mortar and pestle before addition), catalytic I_2, dry THF (20 mL, 0.9M), molecular sieves (flame dried before addition). Stirred at 0°C for 30 minutes. Ice bath was removed, reaction was stirred at room temperature for 40 minutes, no color change occurred. 2,6 dibromo-3,5-dimethoxybenzaldehyde (1 g, 0.4 mmol) was added in dry THF (5 mL, 1 M) at 0°C via syringe and stirred for 20 minutes. Ice bath was removed and stirred at room temperature for 30 minutes. Reaction was stopped, diluted with ethyl acetate and extracted 3x with sodium bicarbonate. The aqueous layers were combined and acidified with 1N HCl until pH was less than 4. Then, they were extracted 3 times with ethyl acetate. Product was recovered from the aqueous layer of the second extraction, diluted with DCM, dried with $MgSO_4$, and concentrated. 38% yield. **IR:** 3521.33, 3503.44, 3427.09, 3391.54, 3382.85, 3368.87, 3337.52, 3317.78, 3288.47, 2930.03, 2855.29, 1701.24, 1649.21, 1331.37, 1216.07, 1085.03

3. Results and Discussion



Scheme 3. Total synthesis of Pestalone analogs where each analog is boxed in

Overall, it was found that the Grignard synthesis procedure was successful for compounds **1.2, 1.4, 1.6, 1.8, 1.10** and **1.13** (Scheme 3), although these reactions resulted in widely variable yields. Each Grignard reaction resulted in the production of an impure product, which required the use of column chromatography to separate the desired product from starting material and other byproducts. At times, very close spotting via thin layer chromatography (TLC) was resulted from the column resulting in a difficult purification process.

Difficulty in the synthesis of product **1.13** was resolved via a modification of the general Grignard procedure that was used for all other bicyclic Grignard reactions. Molecular sieves were added in order to ensure extra protection of the reaction from moisture and the metallic coating of the magnesium turnings was stripped using a mortar and pestle in order to increase the reactivity of the reaction. These precautions were put in place in order to aide the preparation of the ((4-bromophenyl)ethynyl)trimethylsilane for Grignard addition. It is thought that the size of the molecule inhibited its ability to react with magnesium, causing a lack of results in previous trials using the general procedure. Furthermore, an acid-base purification process was performed after the initial extraction, followed by a second extraction in order to circumvent the use of column chromatography. During this workup, both the aqueous layer and organic layer were maintained from each extraction, the aqueous layers being dried to reveal the compounds that they contained. The product was found in the aqueous layer of the second extraction, when TLC showed the appearance of a compound that had a different R_f value than both starting materials. The identity of this product was confirmed with IR Spectroscopy.

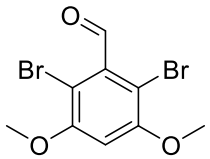
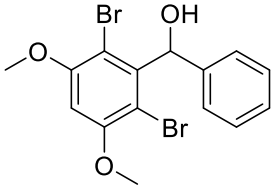
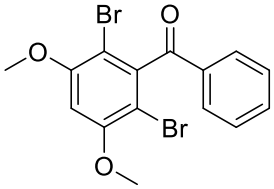
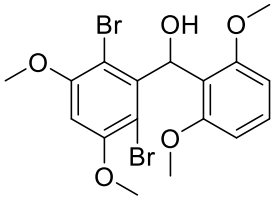
The reaction used to synthesize products **1.3, 1.5, 1.7,** and **1.9** was run at extremely variable times, sometimes demonstrating completion via TLC after 4 hours and often not demonstrating completeness until after being run for

up to 24 hours. Therefore, it was vital to use TLC to check for completeness often during the reaction process, and the use of frequent TLC as well as filtering the reaction before it was complete could have resulted in the loss of product. Often, the reaction would form a thick clay-like biproduct during large scale-ups and this led to difficulty in the filtration process.

The reaction used to synthesize product **1.1** was often high-yielding and successful, and was thus able to be run on a 10.0 g scale at times as opposed to the usual 1.0 g scale to give an increased amount of Grignard reagent for future Grignard reactions in less time. The filtered product was always pure and there was never a need for column chromatography purification.

Although the results of bacterial cell-death assay are ongoing, the results of assays so far seem to indicate antibiotic activity against *S. aureus* for compounds **1.1** and **1.2**. Other bacterial cell-death assay results indicated that compounds **1.3** and **1.6** demonstrated moderate activity against *E. coli* (Table 1). All of these compounds use compound **1.1** as part of their synthesis and products **1.2** and **1.6** were all bicyclic structures that were the direct result of a Grignard reaction. Product **1.3** was the result of the replacement of the alcohol group of product **1.2** with a carbonyl, indicating that further probing of this product's structure promises an increase in antibiotic activity. Compounds **1.2** and **1.3** indicate that the use of a less substituted bromobenzene might increase antibiotic activity. Furthermore, product **1.6** indicates that antibiotic activity was found with an increase in ethers. However, since product **1.7** had no antibiotic activity, it is proposed that the presence of a carbonyl group may counteract the activity of an extra ether group. Compounds **1.11** and **1.12** have not been evaluated for antibiotic activity. However, since both have a much greater magnitude than other compounds synthesized in this work, it is likely that their size may either inhibit the compounds' functional groups from causing cell death, or the large size may be an advantage for each molecule.

Table 1. Compounds that demonstrated antibiotic activity

Compound Name	Compound Structure	Compound Activity
1.1		Activity against *S. aureus*
1.2		Activity against *S. aureus*
1.3		Activity against *E. coli*.
1.6		Activity against *E. coli*.

The results of this research are relevant to a broader area of antibiotic research because they provide a number of synthesized compounds that either have some or no activity that can direct further analysis and modification of analogs of Pestalone. This research also contributes to the field of antibiotic discovery by providing progress in the synthesis of compounds that demonstrate activity against bacteria, which assists in the mission to discover new antibiotics in order to fight the growing issue of antibiotic resistance. Future work in the synthesis of Pestalone analogs should aim

to expand upon compounds found to be active in this study in order to increase their ability to cause cell death of bacteria.

4. Acknowledgements

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