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Synthesis of Pyrazoline Derivatives from Chalcones

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Abstract

Synthesis of pyrazoline derivatives has been an active field of research due to the established biological and pharmaceutical activities of these compounds such as antibacterial, anti-inflammatory, and anticancer properties. Pyrazoline derivatives can be produced from the cyclization of chalcones with hydrazine hydrate and an aryl aldehyde. The goal of this project is aza-Michael addition of aliphatic amines to various α,β unsaturated carbonyl compounds as a novel approach for pyrazoline synthesis using ionic solvents. Previous research has proven that the use of DBU (1,8-diazabicyclo[5.4.0]-undec7-ene-8) as a catalyst/promoter for aza-Michael addition can provide high yields and has the additional advantage of good reusability. However, the "one pot" total synthesis of pyrazoline derivatives has proven to produce low yields. In this project, the reaction between chalcone (**1A-E**), hydrazine hydrate, and benzaldehyde with [DBU][Ac] (1,8-diazabicyclo[5.4.0]-undec7-en-8-ium acetate) acting as a catalyst was found to successfully synthesize various pyrazoline derivatives (**2A-H**). Compounds 2A-D were tested for their biological activity through a series of bioassays (MTT, Cell Death, and XTT). All four compounds possessed both anticancer and antibiotic activity. Compound 2D was found to possess activity comparable to Vancomyocin, a known antibiotic, against gram-positive bacteria. This finding supports the notion that methoxy groups enhance bioactivity. However, further research will need to be completed to test the activity of all pyrazoline derivatives in order to confidently state any trends among biological activity and substituents.

1. Introduction

Cancer prevails as one of the most serious diseases in the world with 14.1 million new cases and 8.2 million deaths worldwide in 2012.[1] Even including the various existing cancer treatment options, cancer remains as an uncontrollable disease. With its rising incidence, new breakthroughs regarding cancer treatments are imperative. The use of organic chemistry to synthesize compounds with anticancer properties may create such breakthroughs. A majority of the research performed by the Holt research group deals with the synthesis of combretastatins, specifically combretastatin A-4 (CA-4) (Figure 1). Combretastatin A-4 is of interest to the research team due to its interaction with tubulin. Microtubules are formed by α-tubulin and ß-tubulin heterodimers. The polymerization and depolymerization of tubulin regulates microtubular dynamics, which play a crucial role in the formation of the mitotic spindle and in cytokinesis at the end of mitosis.[2] Antimitotic agents, or molecules that disrupt microtubule dynamics through binding to tubulins, induce cell cycle arrest and cell death.[2] The taxol/epothilone, vinca alkaloid, laulimalide, and colchicine binding sites are the four binding sites located within the tubulin heterodimer.[2] CA-4 induces cell death by disrupting microtubule dynamics through binding to the colchicine binding site in a tubulin dimer, making it a potentially attractive chemotherapeutic agent.[2] This disruption to the heterodimer and thus inhibition of microtubule polymerization results in the formation of irregular mitotic spindles and cell-cycle arrest at interphase.[2,3] Mitotic inhibition is favorable among anticancer agents since cancer cells undergo mitosis at an increased rate; therefore, cancer cells are more susceptible to mitotic poisoning produced by these agents than normal cells.[3]

Colchicine binding site inhibitors (CSBI) such as CA-4, target tumor vasculature as a mechanism of action since microtubules are vital regulators of endothelial cell biology.[2,3] These agents can either prevent the formation of new blood vessels by outgrowth from preexisting ones (angiogenesis inhibitors), or destroy the existing tumor vasculature (vascular disrupting agents, VDA).[3] These changes in the morphology of the endothelial cells destroy the tumor from within by targeting the core of the tumor. The anti-vascular effects produced by CA-4 differs from other anti-vascular treatments in that other treatments solely target the peripheral cells of the tumor, thus leaving the core unaffected and resulting in the promotion of multi drug resistance (MDR).[2] Therefore, CA-4 is more effective than other treatments in patients that have developed MDR.

Using CA-4 as an antitumor drug is unfavorable due to its poor solubility in biological media and low bioavailability.[4] This information fueled research regarding the synthesis of derivatives of CA-4 with the goal being to enhance anticancer activity by increasing both their bioavailability and solubility in biological media.[4] Various modifications to the substituents of the A- and/or B-ring of the combretastatin were made to enhance anticancer activity.

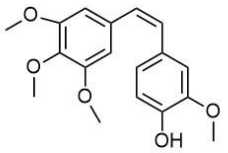


Figure 1. Structure of combretastatin A-4 (CA-4)

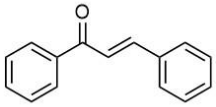
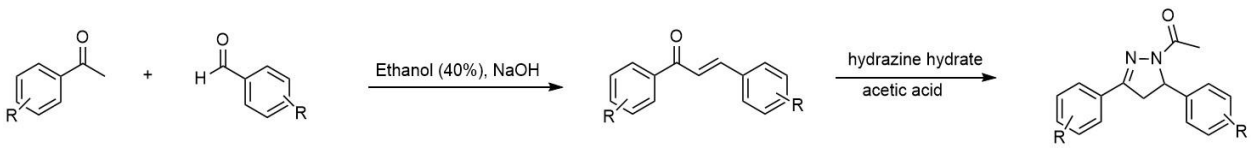


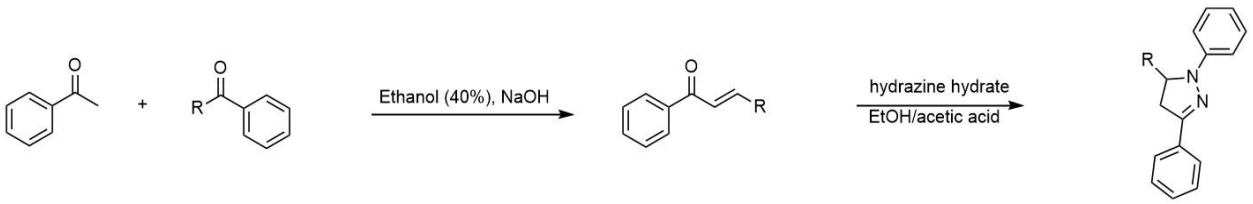
Figure 2. Structure of unsubstituted chalcone

Figure 3. General structure of a 2-pyrazoline vs a 3-pyrazoline

Due to its similarity in structure to combretastatin, chalcone synthesis has been investigated by the Holt lab. Chalcones (Figure 2) are plant-produced flavonoids that are reported to have antibacterial, antifungal, anti-inflammatory, and antitumor responses.[4] The synthesis of chalcone with the addition of substituents to its rings are advantageous when creating compounds with medicinal and pharmaceutical qualities not normally abundant in nature.[4] Chalcones can be cyclized into numerous carbocyclic and heterocyclic compounds. One type of heterocyclic nitrogenous compounds are the pyrazolines (Figure 3). Typically, this cyclization occurs when chalcones are reacted with hydrazine and acetic acid in the presence of ethanol, producing a 2-pyrazoline derivative (Scheme 1).[5,6] Due to their established biological and pharmaceutical activities, pyrazoline synthesis has been an active field of research. For example, pyrazoline derivatives have been previously synthesized using a similar cyclization reaction between chalcones, hydrazine hydrate, and acetic acid to create the derivative shown in Figure 3.[5,6] In both studies, chalcones were reacted with hydrazine hydrate in the presence of cold acetic acid in order to produce their respective pyrazoline derivatives (Schemes 1 and 2). Chalcones have been previously synthesized using a base-catalyzed Aldol condensation in an ethanol solution with sodium hydroxide acting as a catalyst (Scheme 1).[5] However, the Yazdan group's methods for synthesizing 2-pyrzaoline derivatives differed from the Hatwar group.[5,6] The Hatwar research team dissolved a mixture of chalcone and hydrazine hydrate in an ethanol and acetic acid solution before refluxing (Scheme 2).



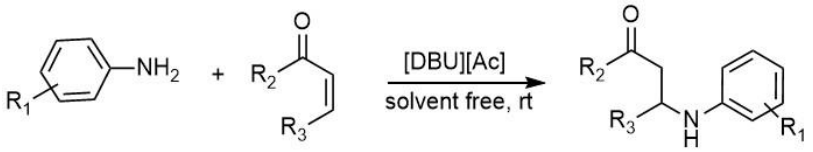
Scheme 1. Synthesis of 2-Pyrazoline derivatives using chalcone, hydrazine hydrate, and glacial acetic acid



Scheme 2. Synthesis of 2-pyrazoline derivatives using chalcone and hydrazine hydrate dissolved in an acetic acid and ethanol solution.

Pyrazoline compounds possess anticancer properties; therefore, the study of various derivatives is imperative to furthering anticancer research. This project focuses on a novel approach to synthesizing pyrazoline derivatives using a solvent-free approach with the ionic liquid, [DBU][Ac] (1,8-diazabicyclo[5.4.0]-undec7-en-8-ium acetate). An ionic liquid is simply a salt in which the ions are poorly coordinated, resulting in the solvents remaining as liquids below 100°C, or at room temperature, otherwise known as room temperature ionic liquids (RTILs).[6] For ionic liquids, at least one ion has a delocalized charge and one component is organic, preventing the liquid from forming a stable crystal lattice.[7] Over the last few years, room temperature ionic liquids have gained attention as possible "green," or environmentally-benign, alternatives to the conventional volatile organic solvents used in reactions.[7] In comparison to organic solvents, room temperature ionic liquids are non-volatile, non-explosive, thermally robust, and potentially recyclable.[7,8] Many ionic liquids have been developed for use in specific synthetic reactions, giving them the name of "designer solvents."[7] This is due to the properties of ionic liquids being determined by the substituents found on their organic component and counterion.[6] The use of task specific room temperature ionic liquids as catalysts for multi-component reactions (MCRs), reactions in which three or more starting materials react to form a product consisting of all or most of the atoms contributing to the newly formed products, is beginning to attract attention.[8] MCRs have already gained considerable attention in the field of medicinal chemistry due to their ability to produce diverse, complex molecules, and coupled with the use of RTILs as a catalyst have been shown to allow some of these reactions to have shorter reaction time, compatibility with a wide range of substrates, and high yields.[8] These important aspects make this particular methodology attractive to further investigation. The reaction at the core of this project can be defined as a "one-pot, three component," or multi-component reaction, therefore the use of an ionic liquid as a catalyst rather than a typical organic solvent could produce results analogous to those aforementioned.

Rather than react a chalcone with acetic acid and hydrazine hydrate in the presence of ethanol as a solvent, this experiment reacts chalcone with benzaldehyde and hydrazine hydrate in the presence of [DBU][Ac] in solvent-free conditions. DBU has been found to be an excellent catalyst/promoter in the aza-Michael reaction of various aromatic amines to α,ß-unsaturated carbonyl compounds (Scheme 3).[9] The aza-Michael addition reaction is a common reaction for the formation of carbon-nitrogen bonds. Reactions of various amines with α,ß-unsaturated carbonyl groups have attracted attention within the chemical field due to their use as anticancer agents.[9] DBU has proven to be a more preferable choice as a catalyst as it does not suffer from the drawbacks associated with strong base and strong acid catalysts. Such drawbacks include the required use of an excess amount of reagents or the substrate selectivity found among some catalysts.[9]



Scheme 3. [DBU][Ac] catalyzed aza-Michael reactions of various aromatic amines with α,ß-unsaturated carbonyl compounds at room temperature.

2. Methodology

2.1 Unsubstituted Chalcone ((E)-1,3-diphenylprop-2-en-1-one)

A mixture containing 5.2 mL of acetophenone, 4 mL of benzaldehyde, 2.145 g NaOH, and 3.8 mL of a water/ethanol (2:1) solution was added to a round bottomed flask. The mixture was stirred in an ice bath for 5 minutes and then stirred at room temperature for 1 hr 20 min. TLC was used to monitor the reaction. The solution was stored in a freezer overnight, producing crystals. The light yellow crystals were vacuum filtered and rinsed with H_2O. The solid product (75% yield) was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 7.4 ppm (t, 2H), 7.5 ppm (d, 4H), 7.6 ppm (d, 1H), 7.75 ppm (d, 2H), 7.79 (d, 2H), 8.1 ppm (d, 1H)

2.2 [DBU][Ac] (1,8-diazabicyclo[5.4.0]-undec7-en-8-ium acetate)

DBU (0.001 mol) was added to a 100 mL two-necked round bottomed flask and cooled by an ice bath ($\leq 5°C$). Glacial acetic acid (0.001 mol) was slowly added to the flask with thorough mixing. After the addition of acid, the ice bath was removed. Due to the reaction mixture being viscous, a condenser was attached and the mixture was stirred on low heat for 24 hours. The 24 hour stir period produced an amber viscous liquid. The residue was dried in vacuo for 24 hours to produce the desired viscous amber ionic "liquid".

2.3 3-(4-fluorophenyl)-1-(4-methoxyphenyl) prop-2-en-1-one

To a 100-mL round bottomed flask was added 0.0016 mol 4-fluorobenzaldehyde and 0.0013 mol of 4-methoxyacetophenone. The starting materials were dissolved in 7.5 mL EtOH. A solution consisting of 0.0080 mol of NaOH and 5 mL of H_2O was added to the flask, causing the solution in the flask to turn golden-yellow in color. The solution was stirred for 10 hr 38 min. The flask was added to an ice bath in order to cool the mixture, producing white, shiny crystals. The mixture was subsequently filtered and rinsed with water and a TLC of the crude product was taken.[9] The crude product (62.51% yield) was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 3.9 ppm (s, 3H), 6.9 ppm (d, 2H), 7.1 ppm (t, 2H), 7.5 ppm (d, 1H), 7.6 ppm (d, 1H), 7.75 ppm (d, 2H), 8.1 ppm (d, 2H)

2.4 3-(4-fluorophenyl)-3-hydrazineyl-1-(4-methoxyphenyl) propan-1-one

To a 25 mL round bottomed flask was added 0.075 g of chalcone, 1.5 mL hydrazine hydrate, and 1 drop of [DBU][Ac]. The solution was stirred for 19 hours at room temperature, turning milky white in color. TLC was used to monitor the reaction, and was stained with ninhydrin to visualize the hydrazine hydrate. The solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL of ethyl acetate. The mixture in the separatory funnel was washed with 1 M HCl (3 x 20 mL) and $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and anhydrous Na_2SO_4 was added as a drying reagent. The organic layer was transferred to a 100 mL round bottomed flask and concentrated through vacuum evaporation, which produced a light yellow oil (22.5% yield). The oil was dried in vacuo and identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 3.3 (d, 2H), 3.9 ppm (d, 3H), 4.6 ppm (t, 2H), 5.4 ppm (s, 1H), 7.61 ppm (s, 2H), 7.51 (d, 2H), 7.75 ppm (dd, 4H), 8.21 ppm (d, 2H)

2.5 Hydrazone

Benzaldehyde (1.5 mmoles), hydrazine hydrate (1.5 mmoles), and 1 drop of [DBU][Ac] were added to a 25 mL round bottomed flask. The color of the reaction mixture turned yellow upon the addition of benzaldehyde. The mixture was stored at room temperature for 16 hours, producing a light amber solution. The reaction was monitored to completion with TLC. The solution was extracted and washed with 1 M HCl (3 x 20 mL) and saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125-mL Erlenmeyer flask and dried with anhydrous Na_2SO_4. The organic layer

was transferred to a 100 mL round bottomed flask and concentrated through vacuum evaporation producing a yellow solid (76% yield). The solid was dried in vacuo and identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 1.0 ppm (s, 2H), 6.89 ppm (s, 3H), 7.2 ppm (d, 2H), 8.12 ppm (s, 1H)

2.6 (3-(4-flurophenyl)-5-phenyl-2, 3-dihydro-1H-pyrazol-4-yl)(4-methoxyphenyl) methanone

(E)-3-(4-flurophenyl)-1-(4-methoxyphenyl) prop-2-en-1-one (0.077 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.5 mL) and benzaldehyde (0.6 mL) were added to the flask. Addition of benzaldehyde caused the reaction mixture to turn bright yellow and temporarily turn cloudy. The mixture was stirred at room temperature for 12.5 hrs. After stirring, the amber reaction mixture was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous $MgSO_4$. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. A yellow liquid/oil residue was formed and stored overnight. The resulting residue was found to be in a yellow solid state (32.9% yield) and was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 3.9 ppm (s, 3H), 4.11 ppm (s, 1H), 4.95 ppm (s, 2H), 7.2 ppm (d, 4H), 7.35 ppm (d, 2H), 7.75 ppm (d, 3H), 8.1 ppm (d, 2H), 8.23 ppm (d, 2H)

2.7 Hexamethoxy Chalcone

To a 100 mL round bottomed flask was added 0.314 g of 3,4,5-trimethoxybenzaldehyde and 0.255 g of 3,4,5-trimethoxyacetophenone. The starting material was dissolved in 5 mL EtOH. A solution consisting of 0.318 g of NaOH and 5 mL H_2O was added to the flask, causing the solution to turn yellow-green in color. The solution was stirred at room temperature for 6 hr 39 min. The flask was cooled by an addition of ice and 2 mL of cold water, resulting in crystal formation. The mixture was then vacuum filtered and rinsed with water. TLC of the crude product was taken. The crude product was recrystallized from a 6 mL 95% ethanol solution, producing a fluffy, bright yellow solid product (88% yield). TLC and ^{1}HNMR of the purified product was taken.

^{1}H NMR ($CDCl_3$): 3.9 ppm (s, 9H), 3.95 ppm (d, 9H), 4.5 ppm (s, 1H), 4.9 ppm (t, 1H), 6.6 ppm (d, 2H), 6.65 ppm (s, 2H), 6.71 ppm (t, 1H), 7.15 ppm (t, 4H), 7.29 ppm (s, 2H)

2.8 (3,5-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)(phenyl) methanone

((E)-1,3-diphenylprop-2-en-1-one) (0.047 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.5 mL) and benzaldehyde (0.4 mL) were added to the flask. Addition of benzaldehyde caused the reaction mixture to turn slightly yellow in color and temporarily turn cloudy. The mixture was stirred at room temperature for 18 hr 20 min. After stirring, the pale amber solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous $MgSO_4$. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. A light yellow liquid/oil residue was formed and stored overnight. The resulting residue was found to be in a yellow-orange solid state (58.6% yield) and was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 4.69 ppm (s, 1H), 5.0 ppm (s, 1H), 5.33 ppm (s 1H), 7.5 ppm (m, 5 H), 7.75 ppm (m, 3H), 8.3 ppm (d, 2H), 8.6 ppm (s, 1H)

2.9 (5-phenyl-3-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1*H*-pyrazol-4-yl)(3,4,5-trimethoxyphenyl) methanone

Hexamethoxy chalcone (0.115 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.4 mL) and benzaldehyde (0.5 mL) were added to the flask. Addition of benzaldehyde caused the reaction mixture to turn yellow and cloudy in appearance. The mixture was stirred at room temperature for 17 hr 4 min. After stirring, the pale yellow solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous $MgSO_4$. The dried organic layer was transferred to a 100 mL round

bottomed flask and concentrated via vacuum evaporation. A light yellow liquid/oil residue was formed and stored overnight. The resulting residue was found to be in a yellow solid state (20% yield) and was identified via ^{1}HNMR.
^{1}H NMR ($CDCl_3$): 4.0 ppm (s, 9H), 4.3 ppm (s, 9H), 5.0 ppm (s, 1H), 5.5 ppm (s, 1H), 7.49 ppm (d, 2H), 7.6 ppm (dd, 4H), 7.67 ppm (s, 1H), 7.86 ppm (d, 2H), 8.6 ppm (d, 2H)

2.10 1, 3 bis (4-methoxyphenyl) prop-2-en-1-one

A mixture containing 1.2 mL of p-anisaldehyde, 1.478 g of 4-methoxyacetophenone, 2.199 g NaOH, and 3.7 mL of a water/ethanol (2:1) solution was added to a 100 mL round bottomed flask. The addition of p-anisaldeyde caused the solution to turn yellow in color. The mixture was stirred in an ice bath for 5 minutes and then stirred at room temperature for 4 hr 5 min, forming a yellow-orange solid. TLC was used to monitor the reaction. The solution was stored in a freezer overnight, producing crystals. The orange crystals were vacuum filtered and rinsed with H_2O. The solid product (89.3% yield) was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 3.9 ppm (s, 6H), 6.95 ppm (d, 4H), 7.41 ppm (d, 1H), 7.78 ppm (d, 2H), 7.92 ppm (d, 1H), 8.04 ppm (d, 2H)

2.11 (4-methoxyphenyl) (3-(4-methoxyphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) methanone

1, 3 bis (4-methoxyphenyl) prop-2-en-1-one (0.082 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.6 mL) and benzaldehyde (0.5 mL) were added to the flask. Addition of benzaldehyde caused the reaction mixture to turn slightly yellow in color and temporarily turn cloudy. The mixture was stirred at room temperature for 17 hr 55 min. After stirring, the yellow-orange solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous $MgSO_4$. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. A yellow-orange liquid/oil residue was formed and stored overnight. The resulting residue was found to be in a yellow-orange solid state (23% yield) and was identified via ^{1}HNMR.

^{1}H NMR ($CDCl_3$): 3.9 ppm (s, 6H), 4.4 ppm (s, 1H), 4.69 ppm (s, 2H), 5.0 ppm (s, 1H), 7.46 ppm (d, 4H), 7.57 ppm (d, 2H), 7.75 ppm (m, 4H), 8.6 ppm (d, 2H)

2.12 3(3-Hydroxy-4-methoxyphenyl)-1-(3, 4, 5-trimethoxyphenyl)-2-propene-1-one

A mixture containing 0.312 g of 3, 4 ,5-trimethoxyacetophenone, 0.221 g of 3-hydroxy-4-methoxy benzaldehyde, 2.002 g NaOH, and 10 mL of a water/ ethanol (2:1) solution was added to a 100 mL round bottomed flask. The addition of the basic solution of NaOH coupled with water and ethanol caused the solution to turn bright yellow then orange, and eventually red in color. The mixture was stirred at room temperature for 4 hrs and monitored with TLC. Water (20 mL) was added to the solution, causing it to turn dark red in color. 1 N HCl was added dropwise to the solution until it reached a pH of 2. The resulting brown solid was vacuum filtered and rinsed with H_2O. The solid was recrystallized using EtOH and stored in a freezer overnight, producing yellow crystals. The crystals were vacuum filtered and rinsed with H_2O. The solid product (16.8% yield) was identified via ^{1}HNMR.

^{1}HNMR ($CDCl_3$): 3.95 (s, 9H), 5.71 (s, 1H), 6.88 (d, 1H), 7.14 (dd, 1H), 7.27 (s, 2H), 7.31 (d, 1H), 7.35 (d, 1H), 7.75 (d, 1H)

2.13 (5-(4-methoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl)-2, 3-dihydro-1*H*-pyrazol-4-yl) (3, 4, 5-trimethoxyphenyl) methanone

Hexamethoxy chalcone (0.116 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (1 mL) and p-anisaldehyde (1 mL) were added to the flask. Addition of p-anisaldehyde caused the solution to turn cloudy and pale yellow in color. The mixture was stirred at room temperature overnight. After stirring, the pale yellow solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous Na_2SO_4. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. A yellow solid was formed and stored overnight. The solid (23.4% yield) was identified via ^{1}HNMR.

^{1}HNMR ($CDCl_3$): 3.9 ppm (d, 21H,), 4.6 ppm (s, 1H,), 5.3 ppm (s, 1H,), 6.9 ppm, (d, 2H), 7.5 ppm (d, 2H,), 8.6 (d, 4H)

2.14 (5-(3, 4, 5-trimethoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl)-2, 3-dihydro-1*H*-pyrazol-4-yl) (3, 4, 5-trimethoxyphenyl) methanone

Hexamethoxy chalcone (0.109 g) and stir bar coated with [DBU][Ac] was added to a 25 mL round bottomed flask. 3, 4, 5-trimethoxyenzaldehyde (0.257 g) and hydrazine hydrate (1.3 mL) were added to the flask. Addition of hydrazine hydrate caused the solution to milky white in color. The mixture was stirred at room temperature overnight. After stirring, the white solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was drained into a 125 mL Erlenmeyer flask and dried with anhydrous Na_2SO_4. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. An amber liquid/oil residue (33%) was formed and identified via ^{1}HNMR.

^{1}HNMR ($CDCl_3$): 3.9 ppm (d, 27H,), 4.6 ppm (s, 1H,), 5.5 ppm (s, 1H,), 6.9 ppm, (d, 2H), 7.65 ppm (d, 2H,), 8.6 (d, 3H)

2.15 (3-(3-hydroxy-4-methoxyphenyl)-5-phenyl-2, 3-dihydro-1*H*-pyrazol-4-yl) (3, 4, 5-trimethoxyphenyl) methanone

3(3-Hydroxy-4-methoxyphenyl)-1-(3, 4, 5-trimethoxyphenyl)-2-propene-1-one (0.01 g) and stir bar coated with [DBU] [Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.5 mL) and benzaldehyde (0.6 mL) were added to the flask, turning the solution yellow in color. The mixture was stirred overnight at room temperature. After stirring, the pale yellow solution was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was then washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was transferred to a 125 mL Erlenmeyer flask and dried with anhydrous Na_2SO_4. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. A yellow liquid (15.2 %) was formed and identified via ^{1}HNMR.

^{1}HNMR ($CDCl_3$): 3.95 ppm (s, 12H), 4.4 ppm (s, 1H), 4.69 ppm (s, 1H), 5.0 ppm (s, 1H), 7.71 ppm (d, 4H), 7.82 (d, 4H), 8.62 ppm (d, 2H)

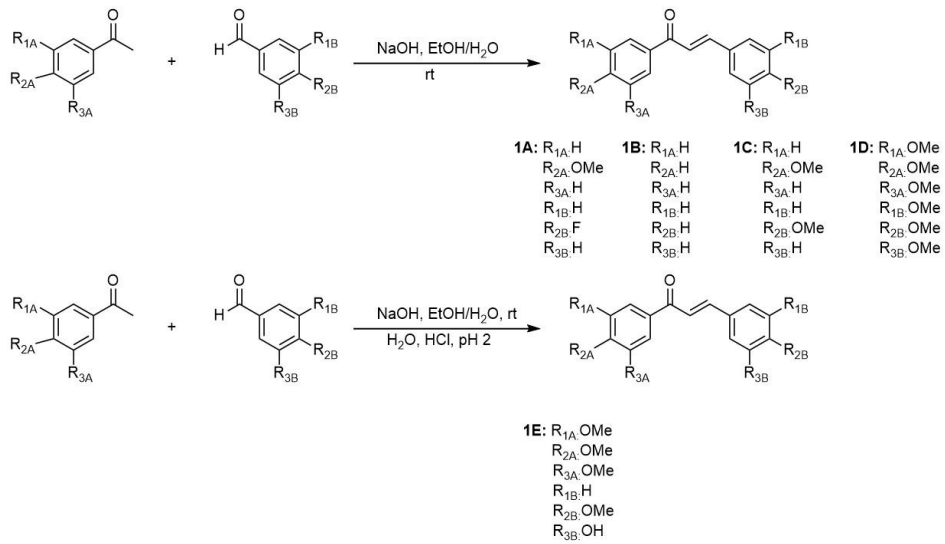
2.16 (3-(3-hydroxy-4-methoxyphenyl)-5-(4-methoxyphenyl)-2, 3-dihydro-1*H*-pyrazol-4-yl) (3, 4, 5-trimethoxyphenyl) methanone

3(3-Hydroxy-4-methoxyphenyl)-1-(3, 4, 5-trimethoxyphenyl)-2-propene-1-one (0.013 g) and stir bar coated with [DBU] [Ac] was added to a 25 mL round bottomed flask. Hydrazine hydrate (0.75 mL) and p-anisaldehyde (0.89 mL) were added to the flask, turning the solution cloudy pale yellow in color. The mixture was at room temperature overnight. After stirring, the pale yellow liquid was transferred to a 125 mL separatory funnel and dissolved in 50 mL ethyl acetate. The solution was then washed with saturated $NaHCO_3$ (3 x 20 mL). The organic layer was transferred to a 125 mL Erlenmeyer flask and dried with anhydrous Na_2SO_4. The dried organic layer was transferred to a 100 mL round bottomed flask and concentrated via vacuum evaporation. Yellow solids (12.4 %) were formed and identified via ^{1}HNMR.

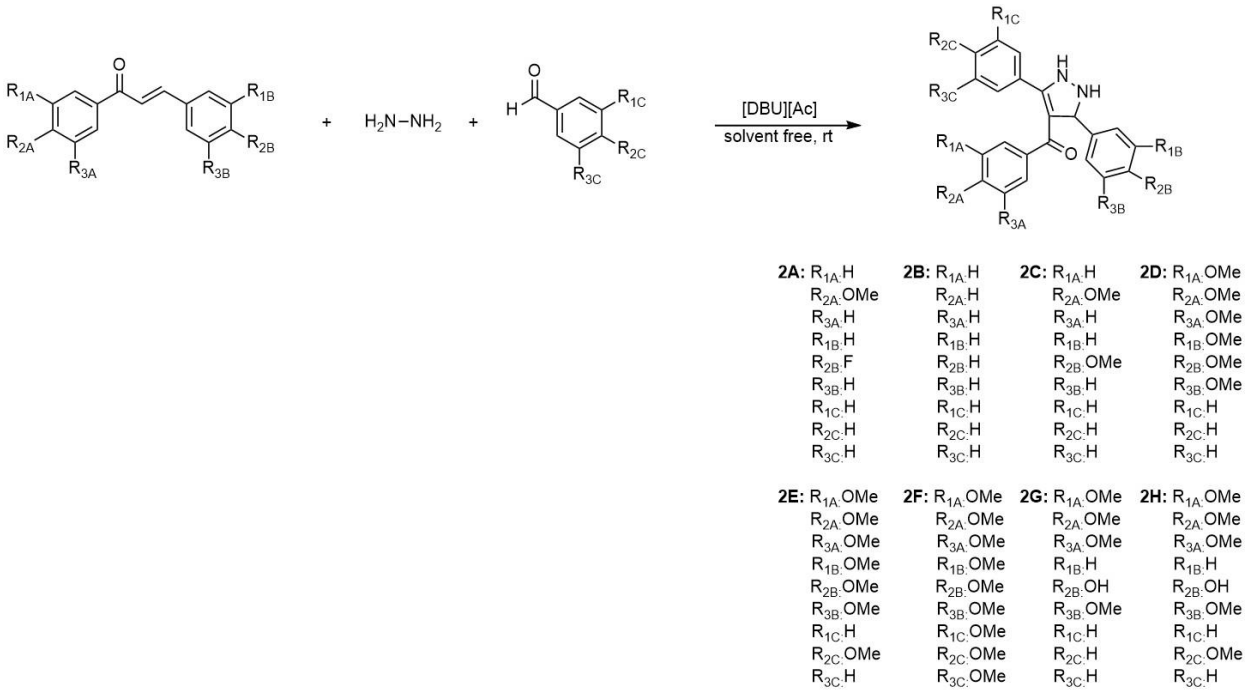
^{1}HNMR ($CDCl_3$): 3.95 ppm (s, 15H), 4.31 ppm (s, 1H), 4.6 ppm (s, 1H), 5.25 ppm (s, 1H), 7.5 ppm (d, 4H), 8.6ppm (d, 3H)

3. Results and Discussion

Chalcone derivatives (Scheme 4) were successfully synthesized and used as starting material to synthesize the pyrazoline derivatives shown in the final synthesis (Scheme 5). An Aldol condensation was performed in order to synthesize the various chalcone derivatives (Scheme 4).



Scheme 4. Synthesis of chalcone derivatives via a base-catalyzed Aldol condensation

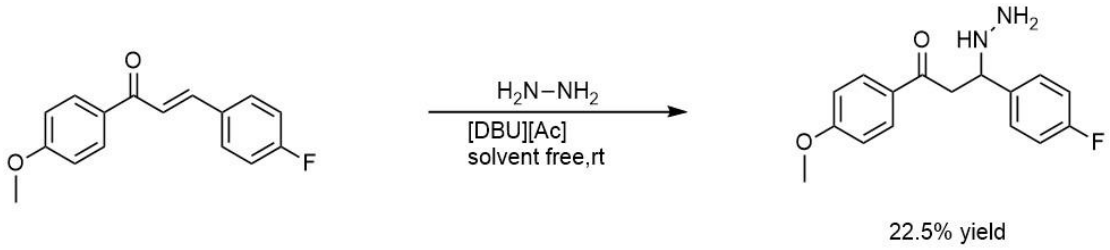


Scheme 5. Final synthesis of pyrazoline derivatives

There were some issues surrounding the initial pyrazoline reactions. The main was the pyrazoline synthesis using chalcone, benzaldehyde, and hydrazine hydrate in the presence of ethanol which was not found in the literature. Therefore, while this reaction works in theory it may not be successful in a laboratory setting. The lack of literature provides an opportunity to embark upon novel chemistry, or could indicate that such a reaction has failed or that it is simply not a popular reaction. The latter may be the case because a majority of pyrazoline syntheses involve the formation of 2-pyrazoline derivatives, involving the use of acetic acid as a cyclizing agent instead of an aryl aldehyde,

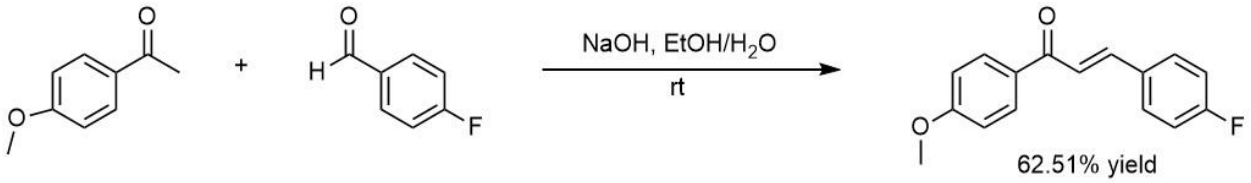
which will not be created in this research project (Figure 3). Synthesis of a pyrazoline derivative from an unsubstituted chalcone under reflux conditions was attempted, but due to a possible contamination of diethyl ether during the extraction phase there was a large loss of product and the pyrazoline derivative was not formed. Another attempt was made without having to complete an extraction, but ^{1}HNMR analysis showed an excess amount of benzaldehyde and a large amount of background noise due to the presence of acetone. After correcting the background through spiking the NMR tube with d-chloroform (to ensure no acetone was present) before adding the sample, it appeared that rather than forming a pyrazoline, the 5-membered, nitrogenous ring of the compound did not close. This suggested that the ratio of hydrazine and benzaldehyde to chalcone needed to be amended in order to obtain a successful pyrazoline synthesis.

Therefore, a reaction involving chalcone and hydrazine hydrate in the presence of [DBU][Ac] was performed (Scheme 6). The purpose of this reaction was to determine how hydrazine hydrate interacted with chalcone without benzaldehyde and what side products formed during the "one pot" reaction if not the desired pyrazoline derivative. From this reaction, it was determined how much hydrazine should be used in the one pot reaction and then the amount of benzaldehyde needed for pyrazoline synthesis was able to be estimated more accurately. The hydrazine hydrate added via a Michael addition proved that [DBU][Ac] could act as a catalyst to both the addition of aromatic and aliphatic amine groups to α,ß-unsaturated carbonyl compounds.

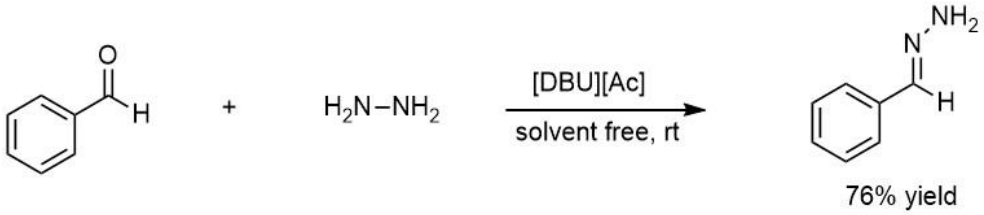


Scheme 6. Synthesis of 3-(4-fluorophenyl)-3-hydrazineyl-1-(4-methoxyphenyl)propan-1-one

To more easily identify the pyrazoline derivative, (E)-3-(4-flurophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one was used as a starting material as both the methoxy and fluorine groups have unique ^{1}HNMR peaks due to *para*-substituted substituents, allowing aromatic signals to be more easily followed. The chalcone derivative was synthesized via an Aldol condensation reaction (Scheme 7). Before attempting to synthesize the pyrazoline derivative, reactions producing anticipated side products from the reaction between chalcone, benzaldehyde, and hydrazine hydrate were performed (Schemes 7 and 8).



Scheme 7. Synthesis of (E)-3-(4-flurophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one via an Aldol condensation

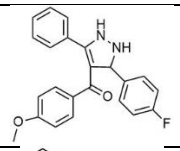
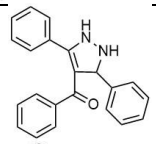
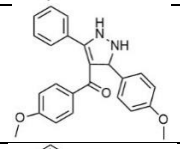
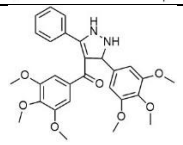


Scheme 8. Synthesis of Hydrazone

The rationale behind creating the possible side products from the reaction between chalcone, hydrazine hydrate, and benzaldehyde was to have ^{1}HNMR data to compare to the pyrazoline derivative's ^{1}HNMR spectrum in order to confidently state that the pyrazoline had been synthesized rather than one of the side products. The pyrazoline derivative while supported by ^{1}HNMR spectra, also had to undergo other identification tests including TLC, IR, ^{13}C-NMR, and LC/MS. The additional identification tests were performed to not only collect data on a novel pyrazoline derivative, but also to prove with the utmost certainty that this derivative was produced from a novel approach with the use of an ionic liquid. However, some difficulties occurred during the preliminary identification testing. It was discovered that the product was temperature dependent, degrading at room temperature after a week. This degradation was apparent due to a color change of the initially yellow product to a dark orange-red. Therefore, the reaction between 3-(4-flurophenyl)-1-(4-methoxyphenyl) prop-2-en-1-one, hydrazine hydrate, and benzaldehyde had to be repeated several times before the final product was stored in a freezer to prevent degradation. Further pyrazoline reactions were successfully performed using the new methodology (Schemes 5).

Compounds 2A-D were tested for their biological activity using the following bioassays: MTT, cell death assay, and XTT. The MTT assay is a common cell viability assay where yellow MTT is reduced to purple formazan in the mitochondria of living cells. Since this reduction only takes place when mitochondrial reductase enzymes are active, conversion can be directly related to the number of viable, or living, cells. The IC_{50} values of the four compounds were calculated from results of the MTT assay (Table 1).

Table 1. IC_{50} values of Compounds 2A-D after incubation periods of both 24 and 72 hours, respectively

Compound	Structure	IC_{50} after 24 hrs	IC_{50} after 72 hrs
2A		49.1 µM	39.9 µM
2B		45.7 µM	49.0 µM
2C		63.7 µM	45.6 µM
2D		64.3 µM	50.8 µM

From the calculated IC_{50} values, Compound 2B is shown to be more effective after a 24 hour period, whereas Compound 2A is more effective after a 72 hour period. However, due to both human error and the possible degradation of the pyrazolines after the 72 hours, the IC_{50} values may not be truly representative of the true anticancer activity possessed by the derivatives. Therefore, the MTT assay will need to be repeated with an emphasis placed on the values calculated from the 24 hour data since the degradation of the compounds will most likely still occur after 72 hours due to their temperature sensitivity.

Similarly to the MTT assay, the cell death assay which measures the effect on drug treatment on bacterial survival, did not produce accurate IC_{50} values. This was due to a lack of data as all compounds showed activity at the highest drug concentration of 1mM (Tables 2 and 3). The compounds were tested against both *Staphylococcus aureus* and *E.coli*. The average absorbance values are represented in the tables and correlate to the amount of viable cells present at each drug concentration. The expectation was that as the concentration of drug decreases, the absorbance values increase, indicating a higher percentage of viable cells. Because definitive conclusions regarding the effects of the various substituents on the antibiotic activity of the pyrazoline derivatives cannot be drawn without IC_{50} values, the

assay may need to be repeated in order to gain the additional data needed to obtain such a value. However, the result of each compound possessing activity is promising and warrants further investigation.

Table 2. Average absorbance values of Compounds 2A-D associated with varying 10-fold dilution concentrations of the respective compound against *Staphylococcus aureus*

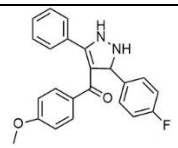
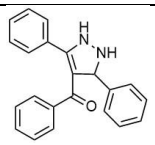
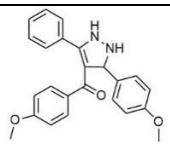
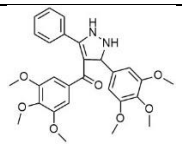
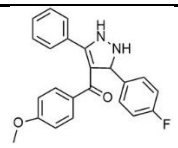
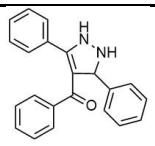
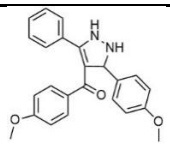
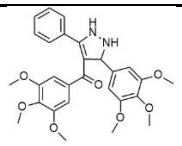
Concentrations (M)	 2A	 2B	 2C	 2D
1000	0.131	0.1305	0.1595	0.4725
100	0.351	0.2145	0.3645	0.34
10	0.3045	0.1895	0.319	0.3265
1	0.3015	0.1925	0.32	0.3205
0.1	0.2935	0.1885	0.3345	0.3215
0.01	0.292	0.1875	0.3325	0.329
0.001	0.307	0.1915	0.339	0.3355
0.0001	0.328	0.1815	0.3325	0.319

Table 3. Average absorbance values of Compounds 2A-D associated with varying 10-fold dilution concentrations of the respective compound against *E.coli*

Concentrations (M)	 2A	 2B	 2C	 2D
1000	0.3855	0.411	0.321	0.4075
100	0.4555	0.4275	0.2215	0.454
10	0.475	0.51	0.4685	0.4835
1	0.5215	0.502	0.469	0.503
0.1	0.479	0.4875	0.4805	0.4545
0.01	0.457	0.4715	0.4725	0.448
0.001	0.4575	0.472	0.503	0.456
0.0001	0.515	0.516	0.5345	0.501

XTT is a colorless or slightly yellow compound that when reduced becomes brightly orange. Similarly to MTT, the bio-reduction only occurs in viable cells; therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture. Compounds 2A-D were tested against four bacterial strains: *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* (Fig. 4). Of interest are the results against the gram-positive bacteria (*Staphylococcus aureus*, and *Bacillus subtilis*). Compound 2D appears to have promising activity against *B. subtilis* as it appears to have activity comparable to Vancomyocin. Typically a XTT assay is run for 24 hours, but due to time constaints this assay was only able to be run for 15 hours. If the assay were to be run for the full 24 hour period, it appears that inhibition against gram-positive bacteria could occur at later points. From this a potential mechanism of action can be deduced. The compounds could behave similarly to Vancomyocin and inhibit cell wall synthesis of the bacteria by degrading the peptidoglycan layer. The compound being active against gram-positive becateria further supports this hypothesis as gram-positive bacteria are more receptive to antibiotics due to the absence of the outer membrance possessed by gram-negative bacteria. The XTT assay will need to be rerun in order to prove this hypothesis.

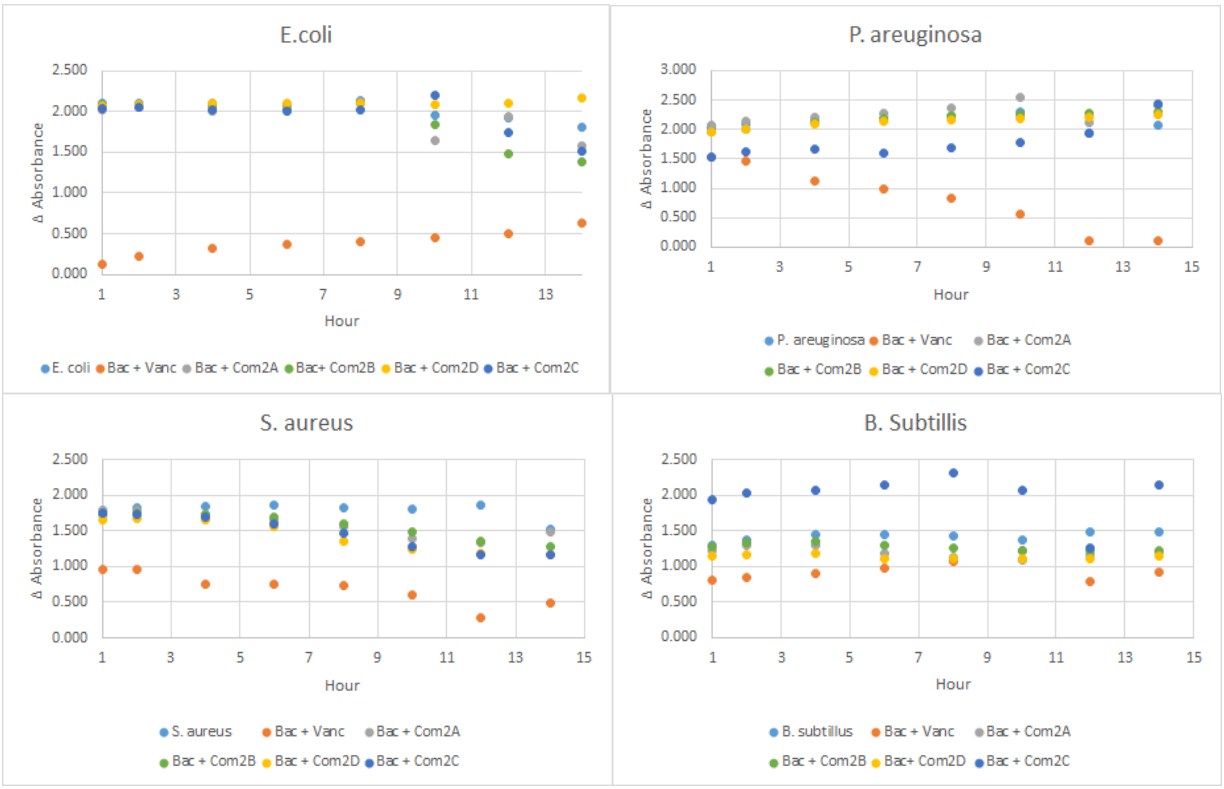


Fig 4. Average absorbance values of Compounds 2A-D during a 13-15 hour time period as a way to test the effectiveness of the compounds against four different types of bacteria. Vancomyocin (Vanc.) was used as a control.

Future research must be completed in order to determine the biological effects of the various substituents on all of these derivatives (Scheme 5) in order to assess whether they have the potential to act as the basis for future antibiotic and/or anticancer agents.

4. Conclusion

In conclusion, aza-Michael addition of hydrazine to chalcone derivatives as a novel approach for pyrazoline synthesis using an ionic liquid was supported by ^{1}HNMR spectra along with the other aforementioned identification tests. Because of this support the use of both an aryl aldehyde rather than acetic acid and an ionic liquid rather than an organic solvent were proven to successfully produce pyrazoline derivatives, providing an entirely new methodology for the synthesis of these particular compounds. Such a reaction may provide the incentive for further investigation into the use of ionic liquids for solvent free conditions in the addition of nitrogenous groups to α,β-unsaturated carbonyl compounds rather than the previous focus on just the addition of aromatic amines to α,β-unsaturated carbonyl compounds with ionic liquids as a catalyst. Future research goals for this project include testing the biological activities of all of the synthesized pyrazolines (Scheme 5) to test the effect of methoxy groups on the biological activity of pyrazoline. This biological activity includes both the anticancer and antibacterial activity of the synthesized derivatives. The synthesized compounds can be evaluated for their anticancer activity against tumor cells in an MTT assay. The results of the bioassays can act as a basis for future modifications to the derivatives to enhance their biological activity.

5. Acknowledgements

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6. References

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