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Synthesis of Pyrrole Molecules as Anticancer Drug Targets

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Abstract

Cancer diagnoses are on the rise and the development of anticancer treatments are becoming increasingly crucial. Leading research in the field of anticancer molecules has focused on antimitotic agents that target the colchicine-binding site of tubulin and halt cell proliferation. The colchicine-binding site has been shown to interact with Combretastatin A4, chalcones, and pyrrole-containing molecules that consist of a five-membered nitrogenous aromatic ring. This research focuses on the synthesis of pyrrole-containing molecules, their ability to inhibit the colchicine-binding site, and their potential to act as DNA cleaving agents. Chalcones will serve as the starting material with glycine ethyl ester as a key reagent to yield the pyrrole-containing molecules of interest. The primary objective of this project is to synthesize a library of chalcones and pyrrole-containing molecules to be examined for antimitotic and antitumor properties using an MTT assay. A dimethoxy chalcone (99% yield), a tetramethoxy hydroxyl chalcone (76% yield), a dimethoxy nitro chalcone (94% crude yield), and a methoxy amino chalcone (74% crude yield) have been synthesized to serve as starting material in the synthesis of various pyrroles. The dimethoxy chalcone, glycine ethyl ester, and N-bromosuccinimide were utilized in a Michael addition reaction, and in a subsequent bromination reaction, to synthesize a brominated pyrrole containing the A and B rings of the dimethoxy chalcone. The tetramethoxy hydroxyl chalcone, structurally resembling Combretastatin A4, was also utilized in a Michael addition reaction and subsequent bromination reaction in an attempt to synthesize the key pyrrole intermediate of interest. In future steps of this research project, this key pyrrole intermediate will undergo a coupling reaction to yield a dienyne substituted pyrrole that will be examined for its potential to act as a DNA cleaving agent.

1. Introduction

A primary focus in the field of anticancer treatments are antimitotic agents which target the colchicine-binding site of tubulin. Tubulin serves as the primary structural component of the microtubules that allow sister chromatids to separate during mitosis. The inhibition of tubulin prevents its polymerization into microtubules and, therefore, prevents cell proliferation.[1] Combretastatin A4 (Figure 1) is naturally derived from the African Bushwillow *Combretum caffrum* and has been found to be a potent colchicine-binding site inhibitor exhibiting antimitotic properties.[2]

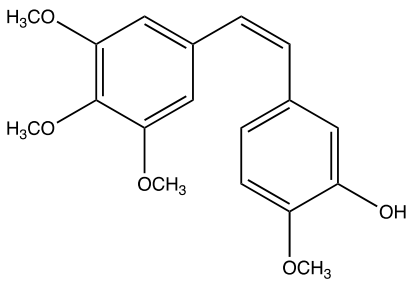


Figure 1. Combretastatin A4.

Similarly to Combretastatin A4, many chalcones can exhibit antimiotic properties by binding to the colchicine-binding site.[1] The colchicine-binding site has been shown to interact with a wide variety of ligands, including pyrrole-containing molecules.[3] Pyrroles, five-membered nitrogenous aromatic rings (Figure 2, pink), are commonly found in naturally isolated products, such as the Polycitones (Figure 2), and have been shown to exhibit cytotoxic effects.[4,5] These pyrrole-containing molecules can be synthesized using chalcones as starting material through a one-pot, three-step synthesis. The rings of the chalcone starting material will serve as substituents to the pyrrole core.[4]

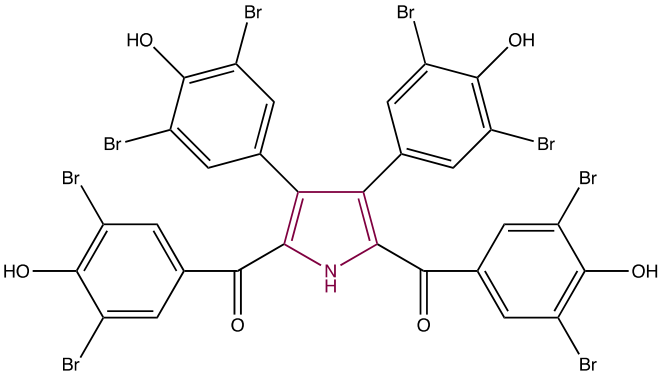


Figure 2. Polycitone B.

A more recent focus in the field of anticancer molecules are DNA cleaving agents that exhibit antitumor effects. The emergence of this field can be attributed to antibiotics such as Neocarzinostatin (Figure 3) and Dynemicin A (Figure 4). These molecules contain an enediyne portion (Figure 4, blue) that can target DNA and undergo reactions that yield diradicals. These diradicals break apart the DNA double helix by deprotonating the sugar phosphate backbone.[5,6]

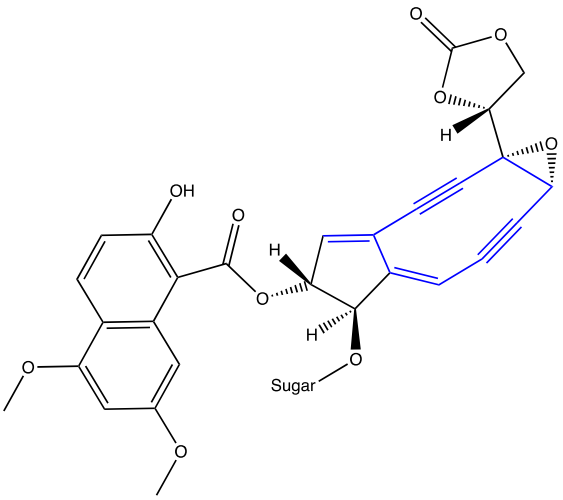


Figure 3. Neocarzinostatin.

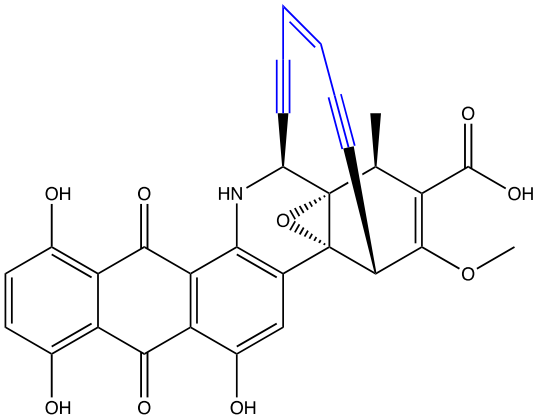


Figure 4. Dynemicin A.

The primary objective of this research project is to create a library of pyrroles derived from chalcones that will be tested against cancer cells to reveal antimiotic effects and structure-activity relationships to the colchicine-binding site of tubulin. The second objective of this research project is to react the pyrrole-containing molecules to form dienynes that can undergo Bergman-like cyclizations to yield diradicals. These diradical molecules are expected to cleave the DNA of cancer cells, acting in the same way as the enediyne antibiotics.

Attaining these goals would further enhance the field of antimitotic and DNA cleaving agents. The results of this project would contribute to prior and current research in the development of chalcones and pyrrole-containing molecules that can bind to the colchicine-binding site. The library of molecules should provide a basis for the

structural moieties to be considered in the design of antimitotic lead compounds. Additionally, studying the ability of pyrrole-containing molecules to act as DNA cleaving agents could reveal critical observations upon which future antitumor lead compounds can be built upon.

2. Background

There has been a heavy amount of research directed towards chalcones as antimitotic agents, thus, the Holt Research Group has recently shifted to examining these pyrrole-containing molecules as inhibitors of the colchicine-binding site. Numerous members of the Holt research group have examined chalcones, while only Malina Navarez and Rogers Muldrow have studied pyrroles and dihydropyrroles. Navarez examined the ability of pyrroles in regards to their antimitotic effects and Muldrow focused on dihydropyrrole synthesis mechanisms.

Gupton et al. focuses on the synthesis of pyrrole-containing molecules, such as the Polycitones. This group has reported on the Suzuki-Miyaura cross-coupling reaction that will be applied to couple an arylalkyne to the pyrroles. Gupton et al. has also reported that the addition of a bromine ortho to the ester gives high yields under these reaction conditions.[7] Therefore, the library of analogs proposed in this research project will contain brominated pyrroles as they are expected to be successful in future synthetic steps in the development of pyrrole-containing molecules. Additionally, the work of Dialer et al., in the synthesis of Lamellarins containing pyrrole-2-carboxylates, will provide the experimental methods for other synthetic steps used to form pyrrole analogues.[8]

Another research group, Ducki et al., focused on chalcones (Figure 5) as colchicine-binding site inhibitors and have utilized docking studies to reveal essential binding moieties. It was determined that the trimethoxyphenyl group on the A ring, the hydroxyl group on the B ring, and the ketone of chalcones are necessary in binding to the colchicine-binding site.[1] The studies by Ducki et al. will guide which chalcones will serve as building blocks of the pyrrole-containing molecules that will be synthesized in this research project.

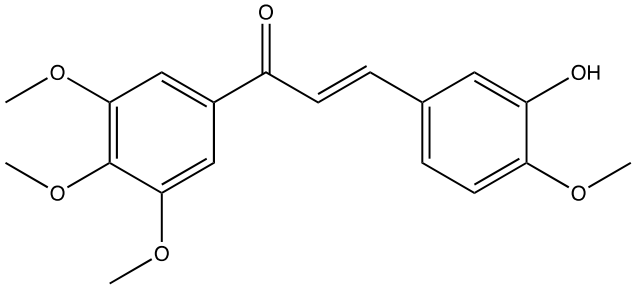


Figure 5. Chalcone analog similar to Combretastatin A4.

In regards to the docking studies carried out by Ducki et al., the AUTODOCK 3.0.5 program was applied to reveal binding modes to the colchicine-binding site. Additionally, in vivo studies were accomplished by MTT assays (discussed in Section 3.3) using a Titretek Multiscan MCC/340 platereader. These in vivo studies conducted by Ducki et al. were used to examine the ability of the synthesized molecules to prevent growth of a cancer cell line. IC_{50} values, measurements that indicate the concentration of a compound needed for 50% inhibition of cell growth, were calculated and compared to analyze the effects of various substituents.[1] A similar approach will be conducted in this research project.

Pyrrole-containing molecules have been studied by Da et al. in regards to the colchicine-binding site revealing the structural-activity relationships of pyrrole-containing ligands. This group reported that an ethyl ester located on C2 of the pyrrole moiety is favored for binding. The pyrrole moiety and its nitrogen atom were also shown to be favorable for binding while methoxyphenyl groups attached to the pyrrole core at C4 also interact with the binding pocket.[3] Based off the structural-activity relationships discovered by Ducki et al. and Da et al., the lead compound of this research project is shown in Figure 6.

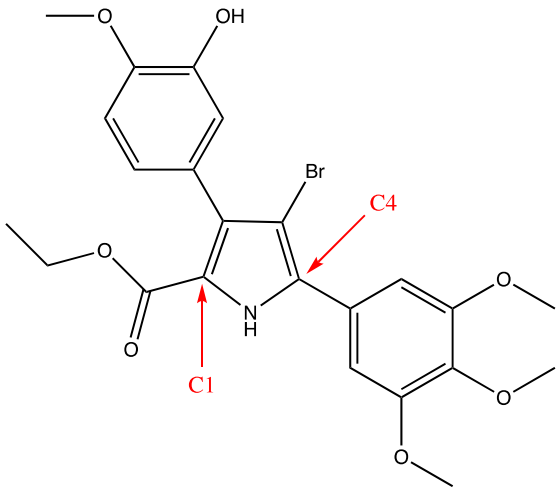


Figure 6. Lead pyrrole compound for M. Wolfe research.

Previous research by Nicolaou et al. focused on DNA-cleaving agents with an emphasis on enediyne-containing molecules. The mechanism of action of these DNA-cleaving agents is initiated by the enediyne group that targets the minor groove of DNA. After a sequence of reactions, cycloaromatization occurs and the enediyne yields diradicals that separate the two DNA strands by abstracting hydrogens from the DNA backbone.[6] The information reported by Nicolaou et al. concerning enediynes will serve as a basis in this research project considering dienynes. After the library of pyrrole analogues are synthesized, they will be coupled to a arylalkyne resulting in a dienyne-containing molecule (Figure 7, blue).[8] The objective is that the resulting compounds can act as DNA-cleaving agents like the enediyne-containing molecules studied by Nicolaou et al.

This research project will build upon the previous chalcone and pyrrole research to create a library of pyrroles derived from chalcones that will be tested for inhibition of the colchicine-binding site. The brominated pyrroles will undergo a Suzuki-Miyaura cross-coupling reaction to add a dienyne substituent to the pyrrole moiety. The resulting dienyne-containing molecule will then be considered for a "Bergman-like" cyclization that will form the DNA-cleaving agent. Each pyrrole molecule, along with each chalcone, will be examined for antimitotic and antitumor properties.

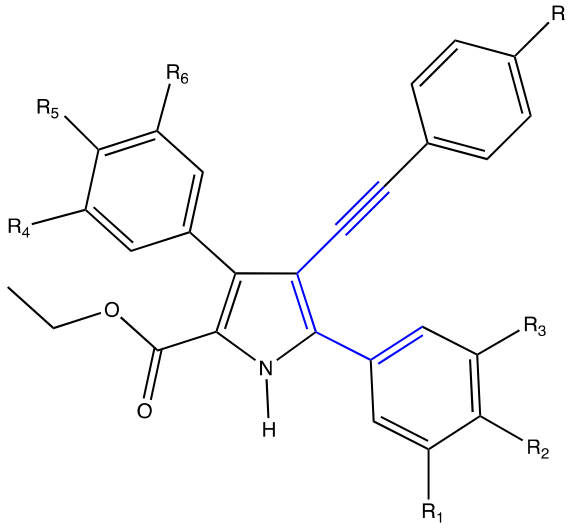
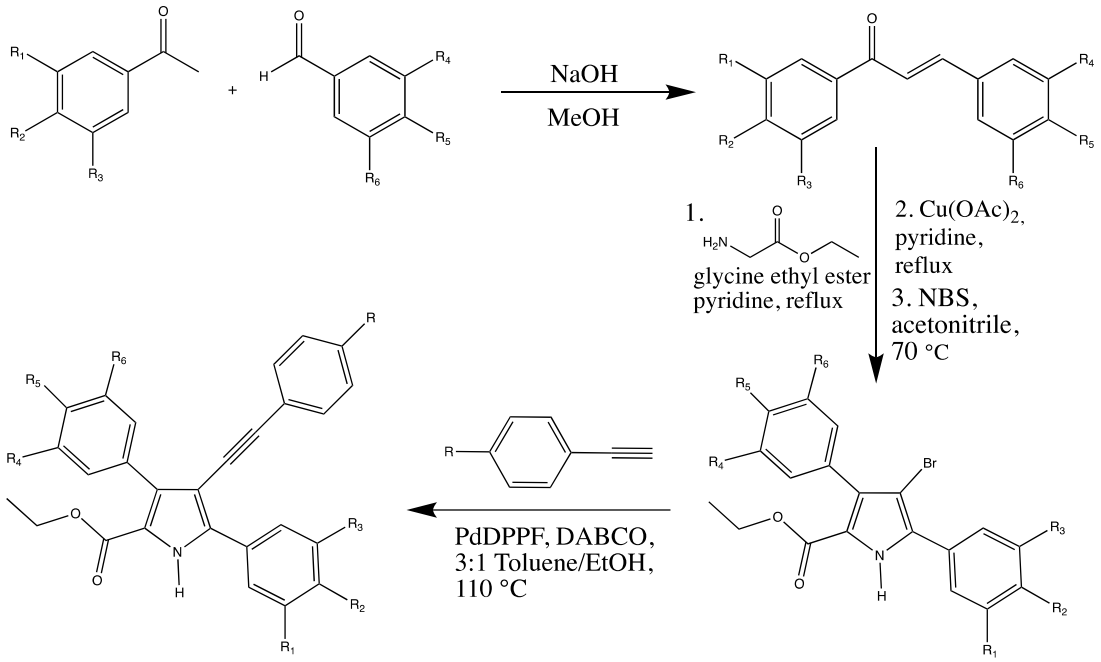


Figure 7. Dienyne-containing lead compound for M. Wolfe research.

3. Results and Discussion

Scheme 1. Anticipated reaction scheme for the synthesis of various chalcones, bromopyrroles, and dienyne substituted pyrroles.[1,8,7]



3.1. Chalcone Synthesis

Claisen-Schmidt reactions were performed to synthesize chalcones (Scheme 1). The synthesis of 4,4'-dimethoxychalcone was successful with a high yield of 99% (Table 1, 1a). The formation of the chalcone product was confirmed by ^{1}H-NMR (Figure 8). The purpose of this synthesis was met as the pure 4,4'-dimethoxychalcone was able to serve as the starting material in the subsequent bromopyrrole synthesis step. Additional chalcones, including 4,4'-dimethoxy-3-nitrochalcone and 4-(dimethylamino)-4'-methoxychalcone (Table 1, 1b and 1c) have also been synthesized. However, these chalcones remain to be purified prior to serving as the starting material in the bromopyrrole synthesis. The synthesis of a 4-(dimethylamino)-3',4',5'-trimethoxychalcone (Table 1, 1e) has also been attempted. However, ^{1}H-NMR indicates that the reaction was not successful, possibly due to an inefficient reaction time.

Attempts to synthesize 3-hydroxy-3',4,4',5'-tetramethoxychalcone (Table 1, 1d) had posed significant challenges. Delays in the synthesis of this chalcone have been accredited to uncontrolled acid-base reactions upon the addition of NaOH and the subsequent addition of H_2SO_4. H_2SO_4 was added to the solution to account for the deprotonation of the hydroxyl group. However, this acid has the potential to protonate in an unrestrained fashion. Therefore, in the most recent attempt to synthesize this chalcone, NH_4Cl served as the neutralizing acid. Additionally, in this attempt, the acetophenone reagent and NaOH were allowed to react prior to the addition of the benzaldehyde reagent. The synthesis of 3-hydroxy-3',4,4',5'-tetramethoxychalcone was confirmed by ^{1}H-NMR (Figure 9) with a 76% yield, similar to the 82% yield reported by Ducki et al. for this product.[1] This chalcone, structurally resembling Combretastatin A4, could then be used in the subsequent bromopyrrole reaction to synthesize the lead pyrrole compound (Figure 6).

The procedure implemented to synthesize 3-hydroxy-3',4,4',5'-tetramethoxychalcone was similar to the methods reported by Ducki et al. for this synthesis. However, this research group added the acetophenone, benzaldehyde, and NaOH concurrently.[1] Based on docking and structural activity relationship studies by Ducki et al., it is anticipated that this chalcone will exhibit antimitotic effects by binding to the colchicine-binding site as it contains the essential trimethoxyphenyl moiety and the hydroxyl group.[1]

3.2. Bromopyrrole Synthesis

A one-pot, three-step reaction synthesis of the expected brominated pyrrole molecule (Scheme 1) was performed. Initial attempts to form the bromopyrrole were unsuccessful though. These unsuccessful results were attributed to the fact that the temperature of the reaction was not reaching the necessary threshold of 125 °C. However, after the temperature issue was corrected, the synthesis attempt using the 4,4'-dimethoxychalcone showed promising results that the bromopyrrole was successfully synthesized (Table 2, 2a). The ^{1}H-NMR spectrum of the crude product revealed a broad signal at approximately 9.4 ppm with the expectation that the signal is indicative of the nitrogenous hydrogen on the pyrrole ring. However, two signals are shown, leading to the idea that brominated and non-brominated pyrrole molecules are present. This is a reasonable conclusion as the N-bromosuccinimide utilized in the reaction was found to contain impurities.

Additional extraction techniques were performed that removed residual solvent and revealed additional ^{1}H-NMR signals that indicate successful synthesis of the bromopyrrole molecule. The following ^{1}H-NMR spectrum revealed a quartet at 4.2 ppm, indicative of the hydrogens adjacent to the ester functional group, and expected signals in the aromatic region. These results further demonstrate the ability of the one-pot, three-step reaction to yield bromopyrroles. However, flash column chromatography is still needed to purify this molecule and a mass spectrometry analysis is necessary to ultimately confirm successful synthesis. Although this bromopyrrole was not yet purified to determine a yield, Dialer et al. reports a 74% yield for a pyrrole molecule resulting from this one-pot, three-step reaction synthesis.[8] After the complete purification and characterization is completed, a Suzuki-Miyaura cross-coupling reaction can be applied to synthesize a dienyne pyrrole molecule and advance to further stages of this research project (Scheme 1).

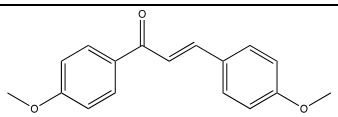
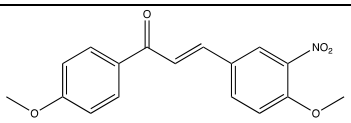
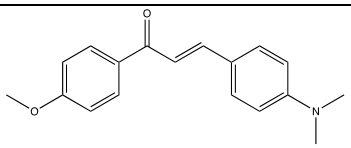
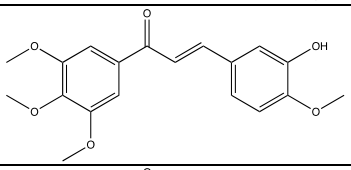
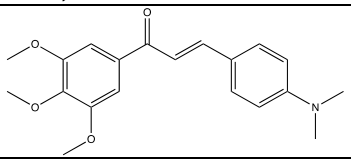
After successful synthesis and purification of 3-hydroxy-3',4,4',5'-tetramethoxychalcone (Table 1, 1d), it was used in the synthesis of the lead bromopyrrole compound (Figure 6; Table 2, 2b). ^{1}H-NMR did not confirm that this pyrrole compound was successfully synthesized. It has been hypothesized that these negative results could be attributed to pyridine's deprotonation of the hydroxyl group of the chalcone starting material. As acidifying the solution did not appear to form the desired pyrrole, the addition of a protecting group to the chalcone molecule to prevent deprotonation of the hydroxyl should be considered. It is also important to note that the temperature of the reaction could potentially be a source of error as it was a few degrees lower than in the previous, and seemingly successful, pyrrole synthesis. Further investigation of the challenges associated with the synthesis of the lead bromopyrrole compound is necessary.

After experimental modifications are implemented and the lead pyrrole compound is successfully synthesized, it is expected to also exhibit antitumor and antimitotic effects. This hypothesis is based on the docking and structural activity relationship studies reported by Ducki et al. and Da et al. Based on these studies, it is anticipated that the lead pyrrole compound will also bind and inhibit the colchicine-binding site as it will contain the essential trimethoxyphenyl moiety, hydroxyl group, and ethyl ester of the pyrrole core.[1,3]

3.3. Biological Analysis

In collaboration with Dr. Jalisa Ferguson, the synthesized 4,4'-dimethoxychalcone was tested against HeLa cancer cells to analyze its cytotoxic activity using a MTT assay. As the MTT compound becomes purple upon reduction to formazan by mitochondrial reductase in living cells, the number of viable cells can be determined using a spectrophotometer to measure absorbance. The MTT assay results indicated that the 4,4'-dimethoxychalcone was inactive against the cancer cells. These results provide a basis for the comparison of the cytotoxic properties of other chalcones and pyrrole molecules. However, it is important to note that these results could be attributed to the low solubility of the chalcone or possibly an inefficient incubation time. Therefore, in the future, the MTT assay should be conducted once more to account for possible sources of error.

Table 1. Mass of reagents, products, and yields in the synthesis of chalcones.

	Synthesized Chalcones	Acetophenone Reagent	Benzaldehyde Reagent	Theorectical Yield	Actual Yield	Percent Yield
1a		1.00 g, 6.66 mmol	1.00 mL, 7.34 mmol	1.787 g	1.779 g	99.6%
1b		1.00 g, 6.66 mmol	1.00 g, 5.52 mmol	1.730 g	1.624 g	94.1% (crude)
1c		1.00 g, 6.66 mmol	1.00 g, 6.70 mmol	1.661 g	1.225 g	74.8% (crude)
1d		1.00 g, 4.80 mmol	0.720 g, 4.80 mmol	1.638 g	1.250 g	76.3%
1e		1.00 g, 4.80 mmol	0.710 g, 4.80 mmol	1.472 g	---	---

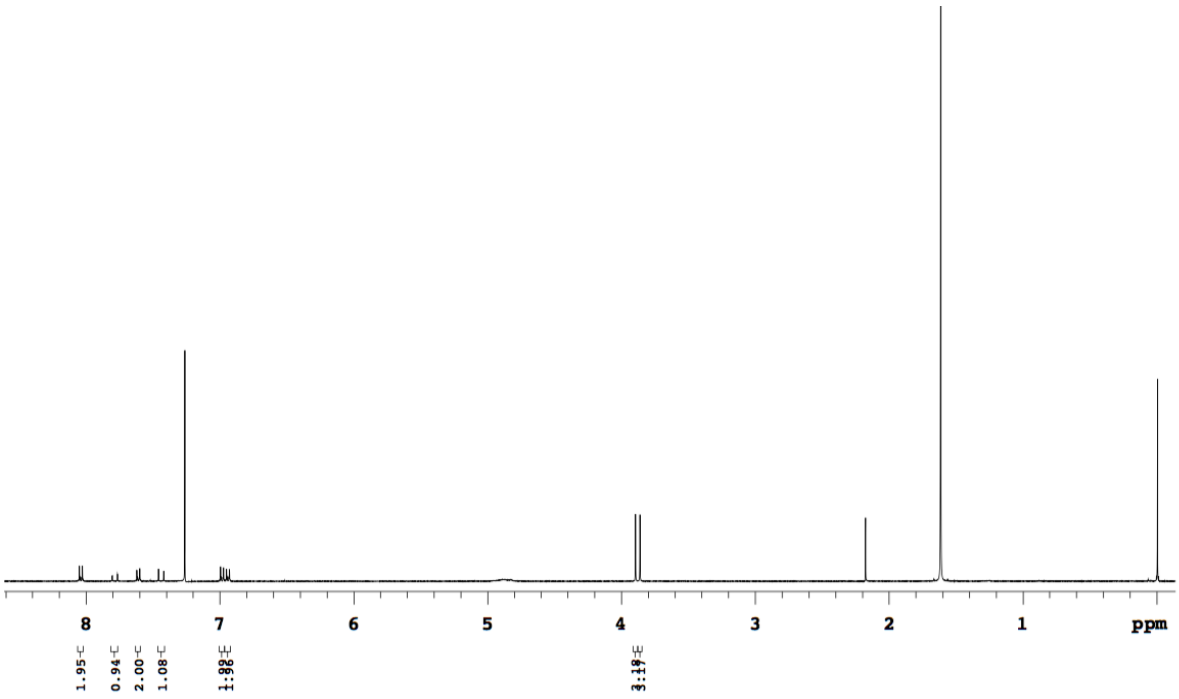


Figure 8. ^{1}H-NMR spectrum of the synthesized chalcone 1a.

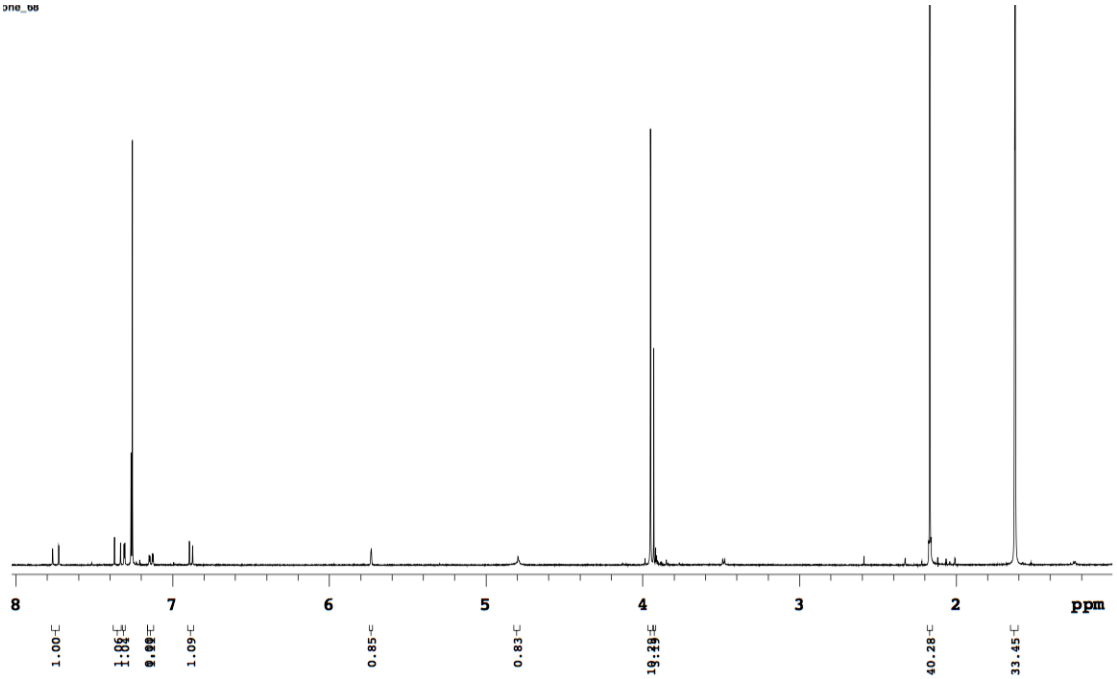
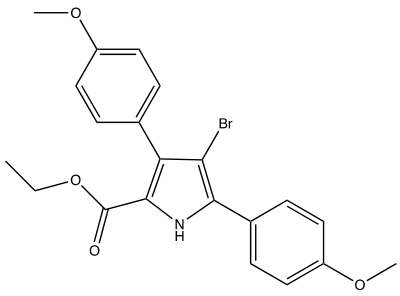
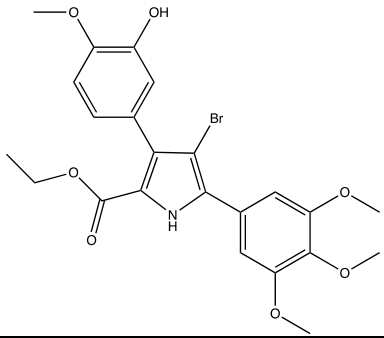


Figure 9. ^{1}H-NMR spectrum of the synthesized chalcone 1d.

Table 2. Mass of reagents, products, and yields in the synthesis of bromopyrroles.

	Pyrrole Synthesis	Chalcone	Glycine ethyl ester	Copper(II) acetate	Halogen Source
2a		0.50 g, 1.86 mmol	0.60 g, 4.00 mmol	0.70 g, 3.70 mmol	0.40 g, 2.30 mmol
2b		0.496 g, 2.90 mmol	0.501 g, 3.59 mmol	0.531 g, 2.92 mmol	0.330 g, 1.85 mmol

4. Conclusion

A 4,4'-dimethoxychalcone (Table 1, 1a) and a 3-hydroxy-3',4,4',5'-tetramethoxychalcone (Table 1, 1d) have been successfully synthesized and purified at 99% and 76% yield, respectively. These yields are reflective of the high yields reported by Ducki et al. in the synthesis of various chalcones.[1] The 4,4'-dimethoxychalcone was inactive against cancer cells. However, this was not an unexpected result as previous studies have shown the importance of the trimethoxy and hydroxyl substituents in inhibition of the colchicine-binding site.[1] In future steps of this research project, each synthesize chalcone should be examined for potential biological activity using the MTT assay.

Experimental results to date indicate the ability of the one-pot, three-step reaction to yield bromopyrroles based on the expected synthesis of the dimethoxy bromopyrrole molecule. However, in the future, this dimethoxy bromopyrrole should be purified and characterized using mass spectrometry to further support this conclusion. Additionally, in the interest of drug discovery, the potential biological activity of this molecule should be examined. In regards to the synthesis of the lead bromopyrrole compound, further investigation is necessary.

In future steps in the progression of this research project, the bromopyrrole molecules should undergo a Suzuki-Miyaura cross-coupling reaction to yield the dienyne-containing lead pyrrole compound. Continued research will involve the analysis of this compound for its ability to act as a DNA cleaving agent, further advancing the field of anticancer drug targets.

5. Experimental

5.1. Synthesis of chalcones

5.1.1 4,4'-dimethoxychalcone (1a)

4-methoxyacetophenone (1.00 g, 6.66 mmol) and 4-methoxybenzaldehyde (1.00 mL, 7.34 mmol) were added to a round bottom flask in methanol (50 mL) with continuous stirring at room temperature. A 15M NaOH solution (10 mL) was added dropwise to the stirring solution to afford a bright yellow homogenous mixture.[1] After TLC confirmed reaction completion, ice was added to the solution to precipitate the product. Solid crystals were filtered using a Hirsh funnel and allowed to dry. ^{1}H-NMR confirmed the pure light yellow compound at 99% yield. ^{1}H-NMR (400 MHz, CDCl3): δ 3.87 (s, 3H), 3.90 (s, 3H), 6.94-6.96 (d, 2H), 6.98-7.00 (d, 2H), 7.43-7.47 (d, 1H), 7.61-7.63 (d, 2H), 7.77-7.81 (d, 1H), 8.04-8.06 (d, 2H).

5.1.2 4,4'-dimethoxy-3-nitrochalcone (1b)

Following the general procedure reported in the synthesis of chalcone 1a, 4-methoxyacetophenone (1.00 g, 6.66 mmol) and 4-methoxy-3-nitrobenzaldehyde (1.00 g, 5.52 mmol) served as the starting materials. The addition of the 15M NaOH solution afforded a dark red reaction mixture and the resulting crystals were a dark yellow. ^{1}H-NMR confirmed the crude product.

5.1.3 4-(dimethylamino)-4'-methoxychalcone (1c)

Following the general procedure reported in the synthesis of chalcone 1a, 4-methoxyacetophenone (1.00 g, 6.66 mmol) and p-dimethylaminobenzaldehyde (1.00 g, 6.70 mmol) were used as reagents. The addition of the 15M NaOH solution afforded an orange reaction mixture and the resulting crystals were a dark yellow. ^{1}H-NMR confirmed the crude product.

5.1.4 3-hydroxy-3',4,4',5'-tetramethoxychalcone (1d)

3,4,5-trimethoxyacetophenone (1.00 g, 4.80 mmol) was added to a round bottom flask in methanol (50 mL) with continuous stirring at room temperature. 15M NaOH (10mL) was then added dropwise to the flask containing only the acetophenone and the solution turned yellow. After at least 1 hour, 3-hydroxy-4-methoxybenzaldehyde (0.720 g, 4.80 mmol) was added to the stirring solution. The solution turned red and was left for 24 hours. NH_4Cl (~25 mL)

was added to neutralize the solution and the mixture turned an orange color. Extractions (4x) were performed with H_2O and ethyl acetate. The organic layer was dried over $MgSO_4$. The solvents were removed under vacuum and ^{1}H-NMR was ran on the product. ^{1}H-NMR indicated successful synthesis of the chalcone. To remove impurities, methanol and ice were added to the solution to precipitate the product. Yellow crystals were filtered using a Hirsh funnel and allowed to dry. ^{1}H-NMR confirmed the synthesis of the pure chalcone at 76% yield.

5.1.5. 4-(dimethylamino)-3',4',5'-trimethoxychalcone (1e)

Following the general procedure reported in the synthesis of chalcone 1a, 3,4,5-trimethoxyacetophenone (1.00 g, 4.80 mmol) and p-dimethylaminobenzaldehyde (0.710 g, 4.80 mmol) were used as starting materials. The addition of the 15M NaOH solution afforded a yellow reaction mixture and the resulting crystals were a bright yellow. ^{1}H-NMR did not indicate product formation.

5.2. Synthesis of bromopyrroles

5.2.1. 6 dimethoxy bromopyrrole (2a)

Glycine ethyl ester (0.60 g, 4.00 mmol) and pyridine (20 mL) were added to a round bottom flask equipped with a reflux condenser. The solution was heated to reflux at 115 °C. The previously synthesized 4,4'-dimethoxychalcone (0.50 g, 1.86 mmol) was then dissolved in pyridine (15 mL) and added dropwise to stirring solution. After at least 24 hours of continuous refluxing and stirring, copper(II) acetate (0.70 g, 3.70 mmol) in pyridine (30 mL) was added dropwise to the refluxing solution. After 4 hours under the same reaction conditions, the reaction temperature was lowered to 70 °C and N-bromosuccinimide (0.40 g, 2.30 mmol) dissolved in acetonitrile (20 mL) was added dropwise to the solution and the reaction continued for another 6 hours. The pyridine was then removed by evaporation and followed by azeotropic distillation with toluene (35 mL).[8] Extractions (3x) with chloroform and water were performed, further purifying the product and removing excess pyridine, and the product was dried under vacuum. ^{1}H-NMR suggested that the desired product was synthesized.

5.2.2 tetramethoxy hydroxyl bromopyrrole (2b)

Glycine ethyl ester (0.501 g, 3.59 mmol) and pyridine (40 mL) were added to a round bottom flask equipped with a reflux condenser. The solution was heated to reflux at 110 °C. The previously synthesized 3-hydroxy-3',4,4',5'-tetramethoxychalcone (0.496 g, 2.90 mmol) was dissolved in pyridine (20 mL) and added dropwise to the refluxing solution. The reaction was allowed to run for at least 24 hours. Copper(II) acetate (0.531 g, 2.92 mmol) in pyridine (30 mL) was then added drop-wise to the refluxing solution. After 4 hours, the reaction temperature was lowered to 70 °C and N-bromosuccinimide (0.33 g, 1.85 mmol) dissolved in acetonitrile (20 mL) was added dropwise to the flask. After the reaction was allowed to run for at least 6 additional hours, azeotropic distillation with toluene (50 mL) was performed to remove solvents.[8] TLC was run on the product to confirm reaction completion. To remove residual pyridine, the product was then washed using water and chloroform (4x) resulting in a dark brown organic layer and a light brown, dense aqueous layer. The organic layer was dried over $MgSO_4$ and solvents were removed under vacuum. Drops of concentrated HCl were added to acidify the aqueous layer and extractions were performed with ethyl acetate (2x) followed by dichloromethane (1x). ^{1}H-NMR did not confirm successful synthesis of the bromopyrrole.

5.3. MTT assay

MTT (24 mg) was dissolved in a phosphate buffer solution (4.8 mL) to create the MTT solution. To create the MTT solvent, HCl (8 mL) was added to isopropanol (500 mL). A 100 mM stock solution of the 4,4'-dimethoxychalcone (25 mg) was prepared using DMSO (930 µL) to dissolve the chalcone.

HeLa cells were cultured in a 96-well plate with approximately 10,000 cells in each well. The media (198 µL) containing fetal bovine serum (10%) was added to the well plate and the incubation period ran overnight at 37 °C with 5% CO_2. Varying concentrations of the 4,4'-dimethoxychalcone were created from the stock chalcone solution to be tested for activity against the HeLa cells. The concentrations of the chalcone were 1 mM, 100 µM, 10 µM, 1

μM, 100 nM, and 10 nM with 1% DMSO and no DMSO serving as the controls. Aliquots (2 μL) of each dilution were added to the 96-well plate. The incubation continued for an additional 22 hours. The MTT solution (50 μL) was then added to each well and, after two additional hours of incubation, the MTT solvent was added to each well (100 μL). A Titretek Multiscan platereader was used to measure the absorbance at each chalcone concentration at 570 nm with a background of 630 nm.[9,10,11]

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7. References

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