University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2015

# Synthesis of Substituted Indole Chalcones

Caitlin M. Bridges
Department of Chemistry
The University of North Carolina Asheville
Asheville, NC 28804 USA

Faculty Advisor: Dr. Herman Holt Jr.

## Abstract

Combretastatin A-4, isolated from the South African bush willow, is well known to bind readily to β-tubulin and disrupt vascular function. Chalcones with structural features similar to Combretastatin A-4 is also known to inhibit tubulin by disrupting its formation or functionality. Several heterocyclic chalcone derivatives have been synthesized in the literature and act as tubulin binding agents. This research examines the synthesis of indole chalcone analogs of Combretastatin A-4 and α-halo substituted indole chalcones utilizing the Hemetsberger-Knittel indole methodology. In the first step, an aldol condensation produced a vinyl azide from a benzaldehyde and ethyl azidoacetate. The product was thermolyzed to produce an indole ester. The indole ester is then reduced with lithium aluminum hydride and subsequently oxidized with IBX to form the indole aldehyde. The indole aldehyde was combined with a substituted acetophenone in a condensation reaction to form the indole chalcone. The structural features of the indole aldehyde are important when reacting with the acetophenones, molecules with ketone functionality. The synthesis of the α-halo indole chalcones utilize the same condensation reaction with the indole aldehyde and an α-halo 3,4,5-trimethoxy acetophenone. The α-halo acetophenone analogs were made through two successive substitutions. All of the reactions produced pure product in 60-80% yield except the final condensation reaction. The indole chalcone synthesis is still causing problems and new synthetic schemes are being explored.

## 1. Introduction

### 1.1 Combretastatin A-4

Natural products have played an important role in drug discovery through medicines and natural poisons for thousands of years. Many common medicines such as morphine and aspirin were derived from plants.[1] A leading drug used in cancer therapy is a natural product isolated from the South African Bush Willow, *Combretum caffrum,* named Combretastatin A-4 shown below.[2] This natural product is classified as an antimitotic agent, a molecule that ceases mitosis by targeting the mitotic spindle.[3] In the treatment of cancer, drugs that target the mitosis process or attack the structure of cells are highly beneficial due to the proliferative nature of cancer cells and tumors.

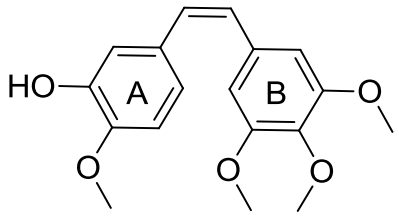



Figure 1. Structure of Combretastatin A-4

The defect in cancerous cells is uncontrolled proliferation due to a failure of checkpoints in mitosis. The integrated checkpoints are supposed to recognize defects in the mitotic spindle or the overall progression of mitosis. Cell proliferation has four phases but two are in place to provide mitotic checkpoints where the cell is analyzed to make sure it has all of the genetic information and structures needed to progress to the next phase.[2] Genetic defects in cancerous cells allow them to bypass the mitosis checkpoints. These checkpoints monitor the mitotic spindle function and determine the ability of the cell to continue mitosis.[3] Since the cells bypass the checkpoints, then they are allowed to continue dividing and making mutant, cancerous, cells.

Microtubules are components of mitotic spindles and are involved in cell proliferation through the process of mitosis.[2] The way the chromosomes are centered in the cell and the shape of the cell are determined by the microtubules. Antimitotic natural products and synthetic drugs in order to affect microtubule formation and in turn mitosis is being explored. Microtubules are composed of α- and β-tubulins stacked through chemical interactions forming heterodimers. These dimers grow outward by the addition of α- and β-tubulins to form a cylindrical structure.[2] The antimitotic agents bind on the side of the heterodimer that faces the lumen of cells. Three natural products—Taxol, vinca alkaloids, and colchicine—have antimitotic properties by binding to tubulin.[4] Mechanistic studies have been conducted on these molecules and other antimitotic agents which revealed that almost all of the compounds target β-tubulin.[3] The trimethoxyphenyl moiety is integral in the binding to β-tubulin due to proximal two cysteine amino acids. Colchicine binds near the α-β interacting face and Taxol binds within the β-tubulin.[2]

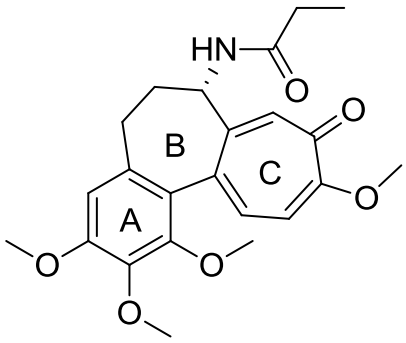



Figure 2. Structure of Colchicine

Colchicine was the first molecule found to inhibit the polymerization of tubulin by binding to what is now termed the colchicine binding site of β-tubulin. The molecule Combretastatin A-4 inhibits the polymerization of tubulin by interacting with β-tubulin at the same site and in the same manner as colchicine. The structure of colchicine is shown in Figure 2. There are two similarities between Combretastatin A-4 and colchicine, 1) the trimethoxy substitution on the A-ring of colchicine corresponds to the B-ring of Combretastatin A-4 and 2) the methoxy functionality the C-ring of colchicine is mimicked via the methoxy on the A-ring of Combretastatin. The alcohol on the A-ring of Combretastatin (Figure 1) mimics the carbonyl functionality on the C-ring of colchicine. Combretastatin A-4 cell studies show that the molecule irreversibly shuts down the vasculature within solid tumors.[6] The shutdown is only in the tumor vasculature and not normal vasculature.[6] The tumors were starved of oxygen and nutrients causing necrosis. Microtubules are integral to the shape of endothelial cell's vasculature. Combretastatin A-4 interrupts the microtubule formation of the young vasculature endothelial cells because the young cells are less developed than mature cells. Once the microtubules are depolymerized, the cells begin to lose the specific shape

they need and instead become round. The swelling of the young endothelial cells constricts the blood flow thus starving the cells of oxygen and nutrients.[6]

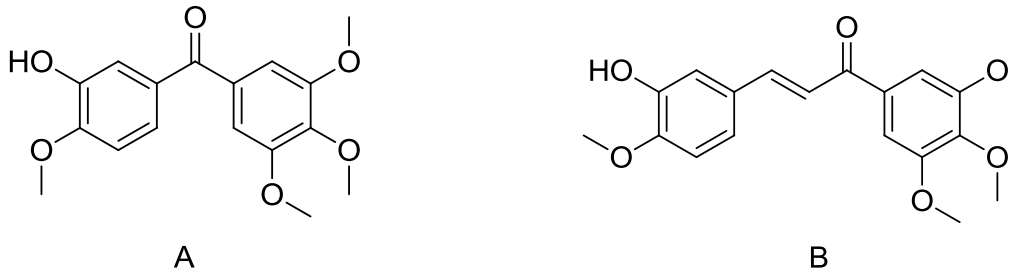



Figure 3. Phenastatin (A) and Chalcone (B) which are Combretastatin A-4 Analogs

## 1.2 Combretastatin Analogs

Advancements in the understanding of the mechanism of Combretastatin A-4 lead to an interest in its analogs (Figure 3) and similar molecules. Combretastatin A-4 has some efficacy problems in vitro due to poor water solubility and pharmacokinetic properties.[2] Pharmacokinetics are the properties of drugs that may be affected by the elements of administration and drug dosage. For this reason, analogs of combretastatin and similar molecules were identified or designed with improved solubility and efficacy. The molecules in Figure 3 were explored in this research—chalcones[5], phenstatins[2], and combretstatins[4]. There have been multiple discoveries of anticancer properties of phenstatins and combretastatins recently but very few in chalcone analogs.

Chalcones have been shown to induce apoptosis in human breast cancer two different pathways, mitochondrial and death receptor.[7] These molecules are possible chemotherapeutic agents to induce apoptosis in cancer cells. Apoptosis is a fundamental cellular activity that is involved in immune defense machinery and has a role as a protective mechanism against carcinogenesis by eliminating damaged or abnormal excess cells that have proliferated.[7] The importance of these anti-cancer molecules is not only apoptosis but also anti-inflammatory, anti-invasive, and antibacterial properties.[8] These effects are often due to the prevention of tubulin polymerization by binding to the colchicine binding site of β-tubulin. The interaction with tubulin is not the only possible anticancer but also antiangiogenic by acting on the tumor vascular. The most cytotoxic derivitives in these studies are trimethoxyphenyl units.[9]

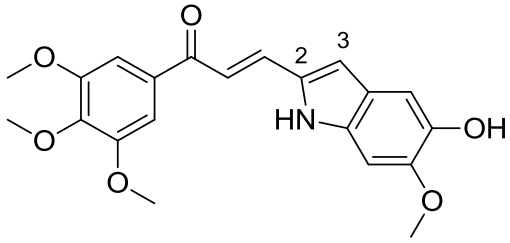



Figure 4. Structure of indole chalcone resembling Combretastatin A-4 substitution with the 2- and 3-positions of the indole ring labeled.

The goal of this research are to synthesize indole chalcone (Figure 4) and to examine their biological efficacy. The efficacy of this molecule could lead to more productive cancer treatments and chemotherapies. The synthetic progress towards indole chalcones will be monitored using nuclear magnetic resonance (NMR), FT-infrared spectroscopy and thin layer chromatography (TLC). The intermediate and final products will be separated from impurities using gravity and automated liquid chromatography. Indoles will be synthesized utilizing a methodology employed by past group members. The strategy to integrate them into chalcones will follow schemes developed by Sylvia Ducki[5] and Nicholas Lawrence[4].

## 1.3 Indole Chalcones

Literature research suggests that no research has been done on indole chalcone molecules substituted at the 2-position of the indole as shown in Figure 4. Utilizing an indole, in place of a benzene ring, could allow for structural stability of the chalcone molecules. Many attempts have been made to retain the cis-olefinic bond of combretastatin by introducing five membered hetero cycle.[8] Ducki et al. completed a multitude of mechanistic, structural and efficacy studies on chalcone analogs.[6] The chalcones were substituted in different manners but none with the indole moiety. They focused on how hydrophilicity and lipophilicity of chalcones aids solubility in order to improve biological efficacy. The addition of the indole poses to improve solubility and biological activity of the chalcone molecule.

Lawrence et al. did extensive work on the synthetic scheme of chalcone formation and fluorinated subtitutions. His group focused on improving the activity and metabolic properties of chalcones.[4] Other researchers have played a role in chalcone research, such as Li and Sham who focused on antimitotic agents, some of them with the indole moiety, that inhibit tubulin polymerization.[2] Li and Sham found that 2-aroylindole derivitives represent another series of promising antimitotic agents that interact with tubulin at the colchicine binding site against microtubule assembly. It was found that the 6-methoxy group on the indole ring is critical and hydrogen on the indole nitrogen are necessary for biological and anticancer properties.[2] Gaikwad et al. did radiation and Quantum studies on an indole chalcone derivative but the alkene carbonyl bridge was attached at the 3-position of the indole instead of the 2-position. They found that the indole added a potent pharmacodynamic nucleus to the chalcone molecule which positively influences the anti-inflammatory activity.[10] Rani et al. explained that heterocyclic moieties substituted at the 3-position show anti-inflammatory, cardiovascular and antibacterial properties. These molecules had increased efficacy but no research on the 2-position.[11] The research focus of this paper is to synthesize indole chalcones with the unsaturated carbonyl linker connected to the 2-position of the indole ring. The effects of the 2-position verses the 3-position will be explored in relation to anticancer, tubulin binding and antibacterial properties.

## 1.4 α substituted Chalcone analogs

In the 1990's, the Edwards' group prepared a series of α-methylchalcones that were found to have ability to halt HeLa cells in mitosis for six hours.[12] This gave strong evidence that α -methylated Chalcones ability to strongly bind to tubulin thus elongating the process of mitosis. One of the most active α -substituted chalcones was found to bind rapidly and reversibility to the colchicine binding site of tubulin. The Ducki group also found that these α-methylated chalcones had powerful antimitotic properties and were able to bind to the colchicine binding site of tubulin.[12] These types of α -methylated chalcones also were reported to have antivascular and anti-inflammatory activity. The data, from Ducki and Edward, suggest that the substitution of the α -position of chalcones can greatly increase the efficacy and bioactivity. Many of the more potent α-substitued chalcones had $IC_{50}$ values in the nanomolar range, which is ideal for drug development. The increase in antimitotic activity has been attributed to the conformation change adopted by the enone backbone. Conformational analysis conducted by the Ducki group showed that chalcones with α-substitution adopt the s-trans conformation, the trans form of the sigma bond located between the carbonyl carbon and the alkene.[12] The s-trans formation of the chalcone has similarities with Combretastatin A-4 than the cis conformation, which should explain the increase in bioactivity and tubulin binding. This could be due to the interaction of the α-position with the binding pocket of colchicine. Thus different substituents, such as halogens, could increase the overall bioactivity of chalcones and even indole chalcones in the α-position.

The Ducki group prepared the α-chloro, α-bromo, and α-fluoro chalcones and found that the α-chloro and α-bromo were the most promising for stopping HeLa from entering mitosis.[12] The most active was the α-fluoro chalcone with the Combretastatin A-4 substitution pattern on the benzene rings.[12] This molecule had an $IC_{50}$ tubulin value in the micromolar range, making it very promising for medical purposes due to the low concentrations necessary.[12] The α-fluoro chalcones also showed significant binding to the colchicine binding site of tubulin. Due to the documentation of high bioactivity, antivascular and anti-inflamatory properties of the α-halogenated chalcones by Ducki and Lawrence lead to the incorporation of halogens into the initial target indole chalcone.

## 2. Results and Discussion

The target molecule in scheme 1(10) can be synthesized from an indole aldehyde and a trimethoxy acetophenone as starting materials. The trimethoxy acetophenone was already available through Aldrich but making the indole aldehyde required many different reactions and techniques. The majority of the project explored the synthesis of indole chalcones and the synthesis of the initial starting materials for the indole aldehyde. Making the indole aldehyde was a multistep process that featured the Hemingberger-Knittel methodology, thermolysis of a vinyl azide to make the indole ester, see Scheme 3.

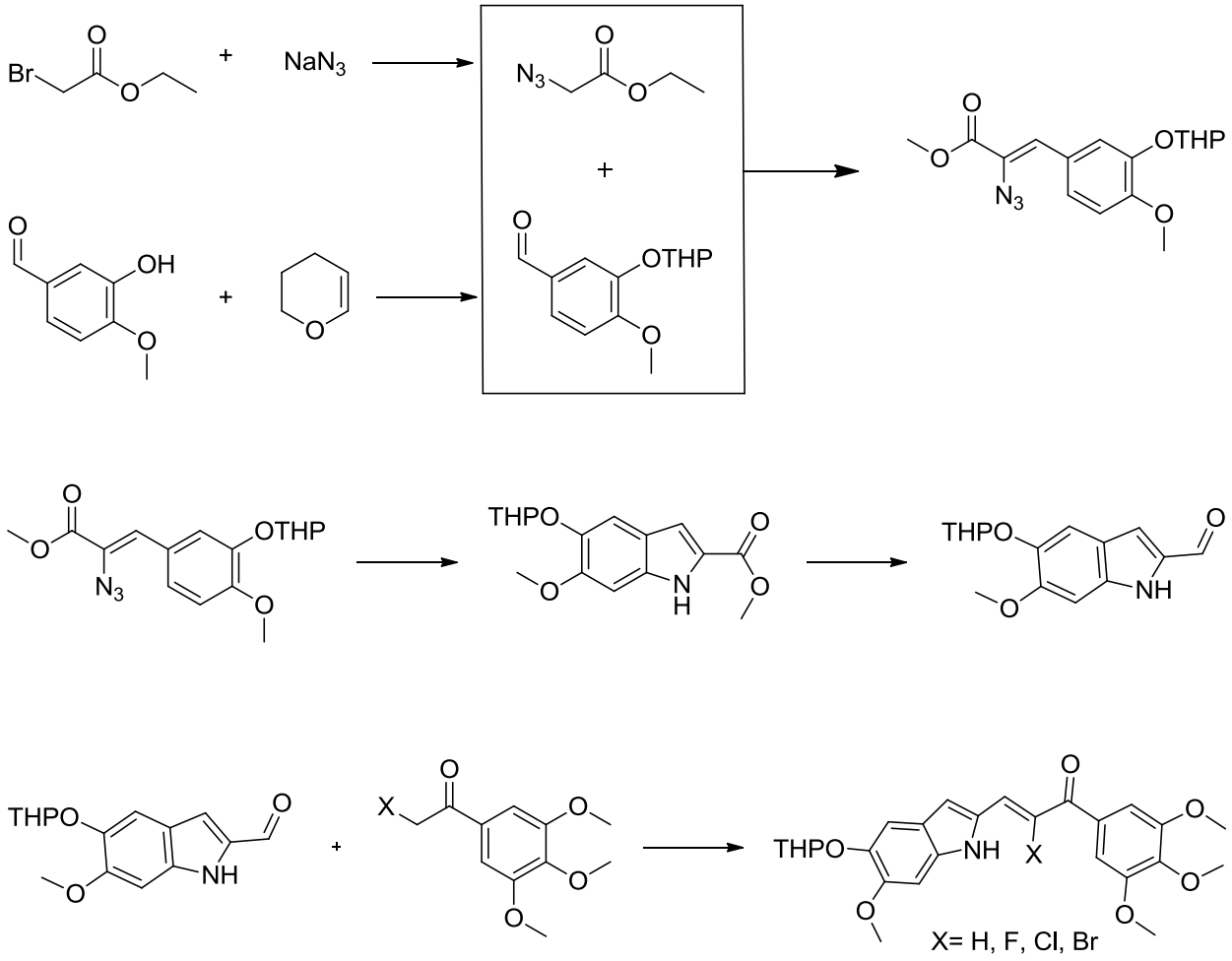
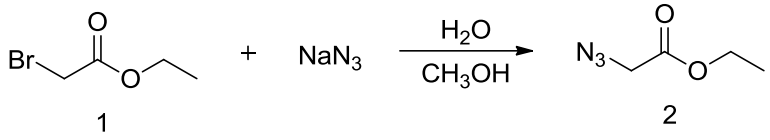



Figure 5. Proposed Indole Chalcone Synthesis Pathway

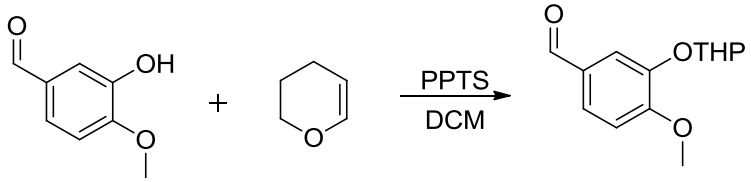
The synthesis of ethyl azidoacetate (2), Scheme 1, showed consistent yields between 60-87% for the trials. The yields remained consistent even when the reactions were scaled up. Two of the five products showed a white solid after concentrating the product under reduced pressure. The pale oil was separated from the white solid by fractionation. The oil continued to the next step even though further NMR examination showed that the white solid and the oil were the same compound.

Scheme 1. Azido Ester Synthesis



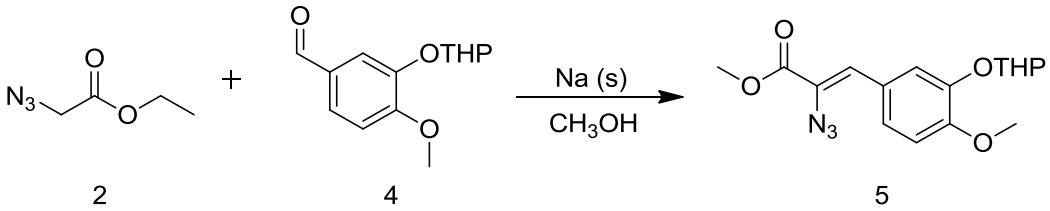

To make the analog of Combretastatin A-4, a hydroxy substituted benzaldehyde was needed. The hydrogen of the alcohol substituent on the benzaldehyde is slightly acidic and can be deprotonated under basic condition in the Hemetsberger-Knittel methodology. Instead of running the risk of the alcohol hydrogen deprotecting, the addition of the 3,4-dihydro-2H-pyran (DHP) was used as a protecting group for the hydrogen, resulting in a tetrahydropyran (THP) protected 3-hydroxy-4-methoxy benzaldehyde (3). The protection of the 3-hydroxy-4-methoxy benzaldehyde, Scheme 2, first trial did not produce a reliable yield or a clean product because the plastic syringe used to add the DHP melted and contaminated the sample. For the remaining three trials, a different brand of syringe was used to prevent contamination of the product. Upon finding an appropriate syringe for using DHP, the reaction proceeded accordingly. Work up of the reaction several $K_2CO_3$ washings to remove any by-products. Condensing the product under reduced pressure formed a viscous pale oil. A solid impurity was observed and removed after being cooled to freezing and the clean oil continued to the next step. $^1$H NMR data was slightly complicated in the 3-4 ppm region because of the THP protecting group but still provided clear evidence that the protected benzaldehyde (4) was formed.

Scheme 2. Protection of Benzaldehyde



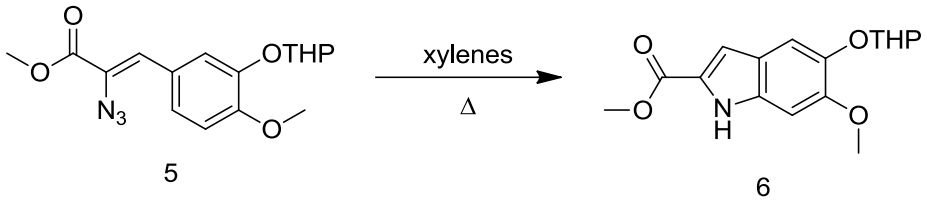

The protected benzaldehyde (4) was subjected to an aldol condensation with ethyl azidoacetate (2) resulted in the the vinyl azide (5) as an orange viscous oil. The condensation showed better yields using temperatures between 10˚C and -10˚C. $^1$H NMR data confirmed that the three trails that were performed produced the same desired compound so the entire mass of the vinyl azide was combined and stored in the freezer immediately. Azides are reportedly light and heat sensitive so the product was quickly stored in a cool and dark environment.

Scheme 3. Vinyl Azide Synthesis



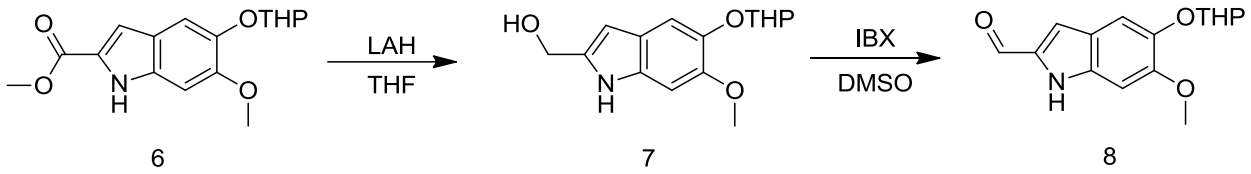

Thermolysis of the vinyl azide (5), Scheme 4, produced the indole ester. When heated the azide underwent an intramolecular rearrangement resulting in the formation of $N_2$ and the production of the indole ring. The accepted mechanism of the Hemetsberger-Knittel which states that the reaction forms an azirine intermediate.[13] The product did not crash out of solution in the initial two trials, as it was supposed to as reported to the literature. The optimum amount hexanes for the recrystallization was found so the following trials did produce a solid precipitate. Filtration afforded the indole ester (6) as a yellow/brown powdery solid. The indole ester was stored in the freezer to keep it from decomposing.

Scheme 4. Hemetsberger-Knittel Indole Ester Synthesis



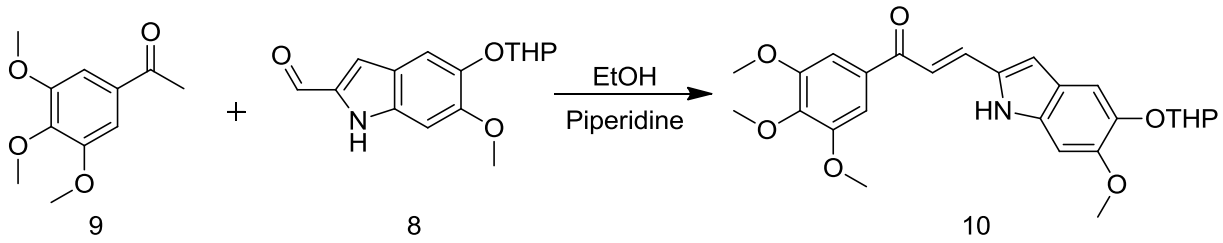

The indole ester (6), Scheme 5, underwent a reduction then oxidation to transform the ester moiety into the desired aldehyde. The reduction of the indole ester afforded the indole alcohol as a brown crystal-like solid. This reaction sometimes produced a brown gummy oil. The oil was then allowed to dry further and ultimately formed a crystalline solid. The synthesis of the indole aldehyde (8) was form by selective oxidation of the indole alcohol (7) using 2-iodobenzoic acid (IBX). This reaction required DMSO as a solvent due to the low solubility of IBX in typical organic solvents. DMSO is readily absorbed through the skin and difficult to remove by reduction under reduced pressure which makes its use more challenging. The reaction was monitored by TLC and after 24 hours showed only faint signs of indole alcohol. The indole nitrogen still showed retention of the hydrogen by $^{1}$H NMR during the conversion. THF was used as a solvent but was not as effective in the conversion of alcohol to the aldehyde. It was later found that the appropriate number of equivalents of IBX to alcohol is critical to the success of the reaction.

Scheme 5. Synthesis of Indole Aldehyde




Having generated the indole aldehyde, the indole aldehyde (8) was reacted with 3,4,5-trimethoxy acetophenone. The attempt to synthesize the indole chalcone (10), involved combining the indole aldehyde (8) and 3,4,5-trimethoxy acetophenone (9) in basic conditions. The basic conditions potentially removed the indole hydrogen so acid was added to give the nitrogen its hydrogen back. $^{1}$H NMR data determined that the reaction did not work, only indole aldehyde and 3,4,5-trimethoxy acetophenone were present.

Scheme 6. Indole Chalcone




The target molecules, Figure 7, required the indole aldehyde (8), previously synthesized, and an α-halo-trimethoxy acetophenone as starting materials. The trimethoxy acetophenone was already available through Aldrich but needed to be functionalized with the appropriate halogen. Making the indole aldehyde used many different reactions and techniques.

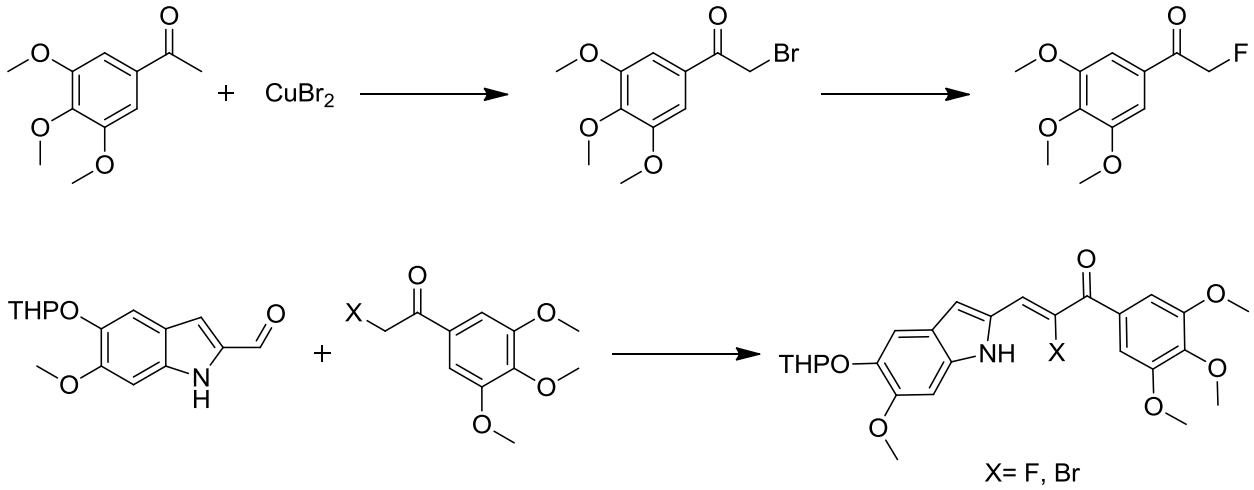
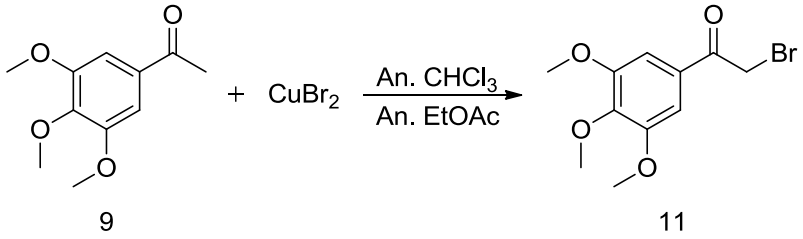



Figure 6. Reaction scheme used to synthesize an α-halo indole chalcones

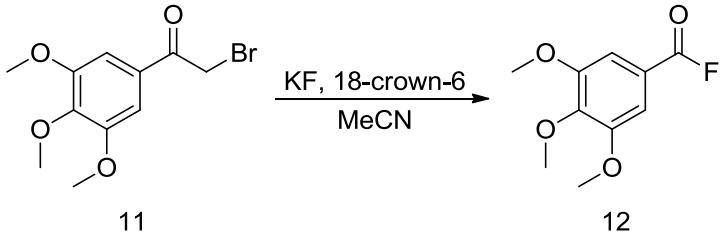
The substitution reaction to form the α-bromo-3,4,5-trimethoxy acetophenone (11), Scheme 7, resulted in a brown solid. The solid contained di-α-brominated and mono-α-brominated products. These two compounds were separated using column chromatography. The equivalents and duration of the reaction were changed to produce more selectivity. When the reaction time and equivalents were increased, there were mono-α-brominated, di-α-brominated and tri-α-brominated. Then the equivalents of $CuBr_2$ were reduced to 1.5 and the reaction time to one hour, which afforded a 7:1 ratio of mono- to di-brominated acetophenone. The monobrominated as the major product, which was easily separated from the small amount of dibrominated via colum chromatography.

Scheme 7. α-Bromo Acetophenone




The substitution reaction to form the α-fluoro-3,4,5-trimethoxy acetophenone (12), Scheme 8, resulted in a pale solid. The solid contained only monofluorinated acetophenone when the monobrominated species was used as the starting material. It was found that when the fluorination reaction began with the mixture of the brominated species the reaction afforded a 5:1 ratio of mono to di fluoro species.

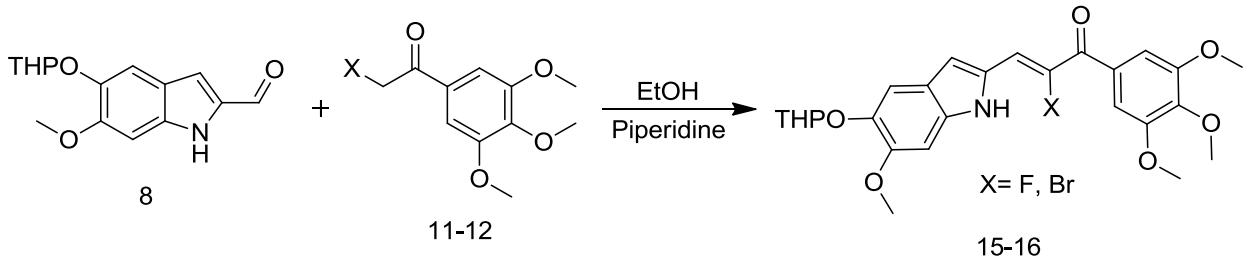
Scheme 8. α-Fluoro Acetophenone




The attempt to synthesize the α-halo indole chalcone, Scheme 9, involved combining the indole aldehyde and α-bromo-3,4,5-trimethoxy acetophenone (11) or α-fluoro-3,4,5-trimethoxy acetophenone (12). The basic conditions of an aldol condensation could remove the indole hydrogen so the Clasien-Schmidt condensation utilizing piperidine as a catalyst was explored. $^1$H NMR data determined that the indole chalcone was not only the product of the reactions.

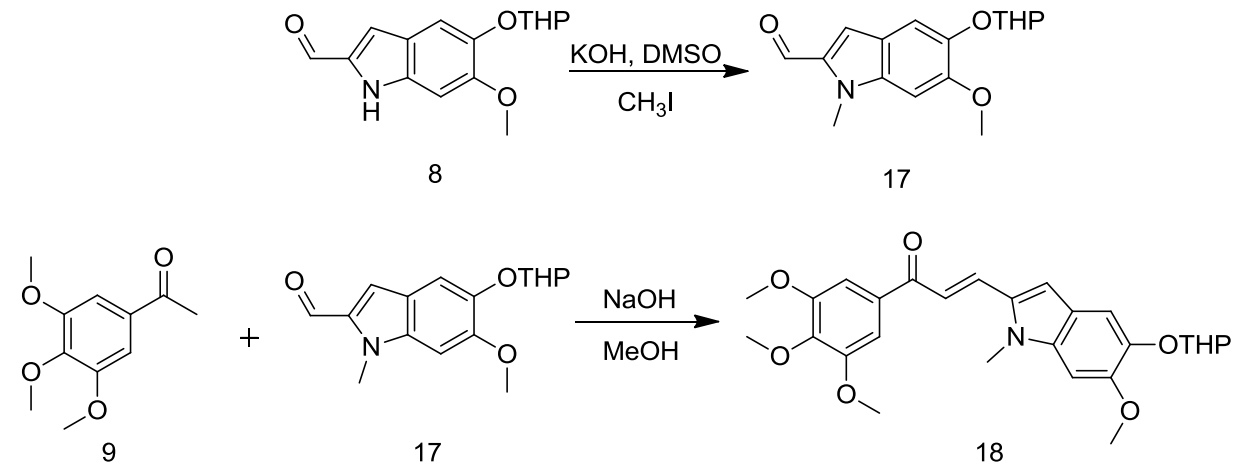
An attempt to isolate the α-halo indole chalcones was made using column chromatography, but the desired product could not be isolated.

Scheme 9. α-Halo Indole Chalcones




Since the indole chalcone and the α-halo indole chalcone reactions did not afford the desired product using basic conditions or a Claisen-Schmidt condensation, the hydrogen of the indole was protected using methylation, Figure 7. Once methylated, the indole hydrogen could not interfere with the reaction conditions of the indole chalcone. The indole aldehyde was methylated, Scheme 10, to protect the nitrogen from deprotonation in aldol condensation conditions. The synthesis of the methylated indole chalcone used with the methylated indole aldehyde (17) and 3,4,5-trimethoxyacetophenone (9) under aldol condensation conditions. $^{1}$H NMR data suggests that the methylated indole chalcone was the product of the reaction.

Scheme 10. Methylated Indole Chalcone




## 3. Conclusion

The purposed of this research has been to prepare the target indole chalcone and α-halo indole chalcones (15-16). The Hemetsberger-Knittel methodology was used to prepare the indole ester in good yields. Subsequent reduction and oxidation reactions provided the desired indole aldehyde (8). The aldol and Claisen-Schmidt condensations utilizing the indole aldehyde and 3,4,5-trimethoxy acetophenone did not produce the desired indole chalcone. The α-halo acetophenone analogs are made through two successive substitutions with reliable yields. The Claisen-Schmidt condensation of the indole aldehyde and α-halo acetophenones did not produce the target α-halo indole chalcone. The indole aldehyde was methylated to avoid any deprotonation in basic conditions of the condensation reactions. The aldol condensation was then attempted with the methylated indole chalcone and 3,4,5-trimethoxy acetophenone. $^{1}$H NMR data indicates the presence of methylated indole aldehyde, 3,4,5-trimethoxy acetophenone, and the desired methylated indole chalcone. Future work will be focused on purifying the methylated indole chalcone (18),

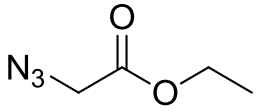
determining an effective and efficient synthetic scheme for producing the indole chalcones, optimizing reaction conditions for the indole aldehyde synthesis and evaluating the compounds prepared for tubulin binding activity.

## 3. Experimental

The NMR spectra were obtained with deuterated chloroform ($CDCl_3$) as a solvent on a Varian Unity INOVA with an Oxford Instruments 400MHz superconducting magnet and Varian INOVA with an Oxford Instruments 500MHz superconducting magnet. A Thermo-Fisher Scientific Nicolet (Madison, WI, USA) iS10 FT-IR spectrometer, equipped with a germanium crystal for attenuated total reflectance was employed for infrared measurements. Spectra treatment and manipulation of data were carried out on Omnic (Thermo Nicolet Corp., Madison , WI, USA) software.

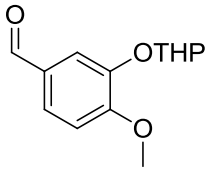
All reagents were purchased from commercial chemical companies. Prior to use in water sensitive reactions, the solvents (THF, DCM, DMSO, xylenes) were dried over activated 3Å molecular sieves and allowed to sit for 8 hours before use. In some cases DCM and THF were extracted from a Pure Solv MD Solvent Purification System equipped with Nitrogen as the inert gas and a 115V, 60Hz vacuum pump. The Acetonitrile was purchased as a dry solvent thus the solvent was kept under nitrogen and no further drying was necessary before use.

### 4.1 ethyl 2-azidoacetate




A 50 mL 3-neck RBF was fitted with a stir bar, a reflux condenser and thermometer. The flask was charged with 20 mL of methanol, followed by ethyl bromoacetate (17.0 mL, 25.7 g, 154.9 mmol) and the mixture was stirred (Caution: Ethyl and methyl bromoacetate are lachrymators, syringes and needles should be left in hood after use). A slurry of $NaN_3$ (7.91 g, 120 mmol) and 13 mL of $H_2O$ was then added via funnel. The suspension was allowed to stir at 70 ºC overnight. A pale tan/yellow color marks progress of reaction, and becomes darker over time. Upon cooling to room temperature, the reaction mixture was poured over 60 mL of $H_2O$. This mixture was washed with 90 mL of diethyl ether and the ethereal extracts removed. The organic layers were dried with $Na_2SO_4$ and concentrated under reduced pressure to give the product as pale tan-yellow oil (17.4 g, 135 mmol, 87% yield). The product was a pale oil $^1H$ NMR ($CDCl_3$): δ 4.27 (2H, q), 3.88 (2H, s), 1.32 (3H, t).

### 4.2 4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)benzaldehyde



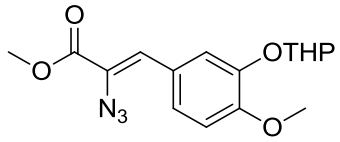

A 250 mL two neck RBF was flame dried, cooled under a stream of nitrogen and fitted with a magnetic stir bar and rubber septum. The flask was charged with 200 mL of anhydrous DCM and 3-hydroxy-4-methoxybenzaldehyde (6.44 g, 42.3 mmol) followed by a catalytic amount of pyridinium p-toluenesulfonate (PPTS) (1.59 g, 6.35 mmol) The resulting mixture was sirred until complete dissolution occurred. 3,4-Dihydro-2H-pyran (DHP) (23.2 mL, 254 mmol) was added to the mixture dropwise via syringe. The reaction mixture stirred at room temperature for 2 hours and TLC indicated the presence of starting material so an additional portion of DHP (1.00 mL, 0.920 g, 10.9 mmol)

was added dropwise via syringe. After an additional hour of stirring TLC indicated the reaction had gone to completion and the mixture was concentrated under reduced pressure. The concentrate was then washed with 200 mL of water and extracted with 2x100 mL of diethyl ether. The combined organic layer were dried over sodium sulfate and then concentrated under reduced pressure to afford the protected benzaldehyde. The product was a viscous pale oil (7.35 g, 31.11mmol, 73.5% yield ) $^1$H NMR ($CDCl_3$): δ 9.80 (1H, s), 7.64 (1H, s), 7.49 (1H, dd), 6.96 (1H, d), 5.45 (1H, t), 3.95 (3H, s), 3.65 (2H, m), 2-1.5 (6H, multiple different peaks).

### *4.2.1 Pyridinium p-toluenesulfonate*

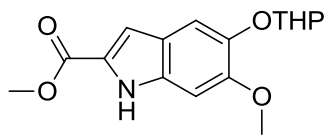
A 25 mL RBF was fitted with a magnetic stir bar. The flask was charged with p-toluenesulfonic acid (2.60 g, 13.7 mmol) and pyridine (5.52 mL, 5.42 g, 68.5 mmol). The reaction was allowed to stir for 30 minutes. The excess pyridine was removed via rotary evaporation. The solid was recrystallized in acetone then filtered using a fritted funnel. The product was afforded as a pale yellow crystal (0.94 g, 3.74 mmol, 94% yield).

## 4.3 (Z)-methyl 2-azido-3-(4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)acrylate




A 50 mL 2-neck RBF was fitted with a stir bar, reflux condenser and addition funnel then oven dried. The entire set-up was cooled under dry $N_2$ and then the flask chilled in an ice bath. The chilled flask was charged with 5 mL of anhydrous MeOH via syringe and 0.370 g of sodium metal (16.1 mmol, 3.27 eq.). The white-yellow slurry was allowed to stir until the sodium metal was completely dissolved. The sodium methoxide/ methanol mixture was cooled further to -10 ºC using a methanol/dry ice bath. In a separate oven dried flask 1.5 g of ethyl azidoacetate (11.6 mmol, 2.36 eq.) and 1.16 g of protected benzaldehyde (4.92 mmol, 1.00 eq.) were dissolved in 4.00 mL of dry methanol. This mixture was added dropwise to the sodium methoxide/methanol mixture over 1-2 hours and the reaction mixture remained at -10 ºC for 4 hours. The reaction mixture turned a pale yellow color that gradually darkened over time. After 4 hours the reaction was allowed to stir overnight and gradually warm to room temperature. The completion of the reaction was verified by disappearance of 3,4,5-trimethoxybenzaldehyde on TLC. The resulting yellow reaction mixture looked to have the presence of a pale yellow or white precipitate. The reaction was poured over 50 mL of a crushed ice/aqueous sat. $NH_4Cl$. The mixture was then washed with 40 mL of diethyl ether and the ethereal layer removed. The organic layers were dried over $Na_2SO_4$ then condensed under reduced pressure to give the product as a viscous orange oil (0.804 g, 53.6%). $^1$H NMR ($CDCl_3$): δ 8.70 (1H, s), 7.36 (1H, s), 7.10 (1H, s), 6.84 (1H, s), 5.35 (1H, t), 3.92 (3H, s), 3.91 (3H, s).

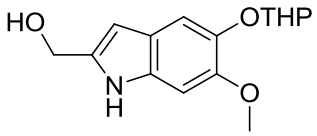
## 4.4 methyl 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylate[13]




A 250 mL 3-neck RBF fitted with a reflux condenser and addition funnel were oven dried and then cooled under a stream of nitrogen. The flask was then fitted with a thermometer and stir bar. The flask was charged with 300 mL anhydrous xylenes and was brought to reflux at 140 ˚C. The vinyl azide (6.97 g, 32.8 mmol) was dissolved in 30 mL of anhydrous xylenes. The vinyl azide solution was added to the refluxing xylenes using an addition funnel set to a slow drip. The addition was completed over the next hour. The solution of xylenes turned orange after the addition

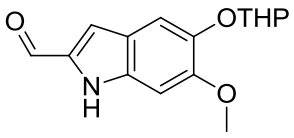
of the vinyl azide. After the reaction refluxed overnight, the TLC confirmed that none of the starting material was still present. The reaction mixture was concentrated under reduced pressure until approximately 50 mL of solution remained. Hexanes were added dropwise to the remaining solution until the product began to crash out of solution. The solution of hexanes and xylenes was crystallized in the freezer for 8 hrs. After crystallization the solution was vacuum filtered using a fritted funnel, affording the indole ester. The product was a brown crystalline powder (4.21 g, 13.7 mmol, 42% yield) $^1$H NMR ($CDCl_3$): δ 8.70 (1H, s), 7.36 (1H, s), 7.10 (1H, s), 6.84 (1H, s), 5.35 (1H, t).

## 4.5 (6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)methanol




A 2-neck 25 mL RBF was fitted with a magnetic stir bar, flame dried and cooled under a stream of nitrogen. The flask was charged with lithium aluminum hydride (0.334 g, 8.80 mmol) and 10.0 mL of dry THF, placed in an ice water bath and set to stir. In a separate flame dried 1-neck RBF Methyl 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylate (1.00 g, 3.27 mmol) was dissolved in 5.00 mL of dry THF and added to the reaction flask dropwise via syringe. Upon addition, an exothermic reaction was observed. The reaction mixture was allowed to stir for an hour after which TLC confirmed complete consumption of starting material. The reaction mixture was then diluted with 20.0 mL of diethyl ether and 3.00 mL of saturated $NH_4Cl$ was added dropwise via syringe (Note: Sat. NH4Cl was added until no vigorous reaction was seen). The reaction mixture was filtered through a course fritted funnel and rinsed with 2x20 mL of diethyl ether. The organic extracts were collected and concentrated under reduced pressure to afford the title compound as a yellow powder (0.820 g, 2.96 mmol, 90% yield) which was used without any further purification. $^1$H NMR ($CDCl_3$): δ 8.21 (1H, s), 7.20 (1H, s), 6.78 (1H, s), 6.21 (1H, s), 5.25 (1H, t), 4.68 (2H, s), 3.80 (3H, s), 3.65 (2H, m), 2.1-1.8 (6H, multiple peaks).

## 4.6 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde



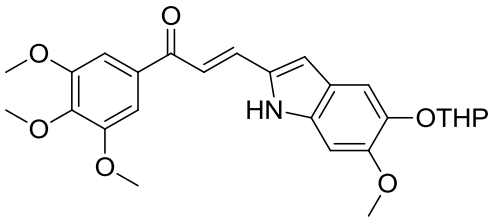

A 1-neck RBF was fitted with a magnetic stir bar and charged with (6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)methanol (0.820 g, 2.96 mmol) in one portion followed by 60.0 mL of anhydrous DMSO. IBX (1.82 g, 6.51 mmol) was added and the reaction mixture was allowed to stir over night (~18 hrs). TLC confirmed that the reaction had gone to completion and the reaction mixture was diluted with 60 mL of ethyl acetate and 60 mL of saturated $NaHCO_3$ (pH ~ 8) and gravity filtered through a pad of celite to remove excess IBX. The filter was rinsed with 60 mL of ethyl acetate. Aqueous and organic layers were separated and the aqueous layer was extracted with 2x30 mL of ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford the title compound as a dark brown residue (0.51 g, 1.85 mmol, 63% yield). $^1$H NMR ($CDCl_3$): δ 9.61 (1H, s), 9.05 (1H, s), 7.33 (1H, s), 7.09 (1H, s), 6.77 (1H, s), 5.30 (1H, t), 3.85 (3H, s), 2.1-1.8 (6H, multiple peaks).

### *4.6.2 2-iodoxybenzoic acid*[14]

A 3-neck 500 mL RBF was fitted with a magnetic stir bar, flame dried and cooled under a stream of nitrogen. The flask was charged with 2-iodobenzoic acid (34.7 g, 140 mmol) and oxone (86.1 g, 140 mmol) and then heated to 70

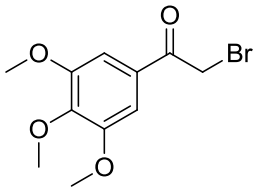
˚C. The reaction mixture was allowed to stir for 3 hour after which TLC confirmed complete consumption of starting material. The reaction mixture was then allowed to cool to room temperature and then placed in the refrigerator overnight. Then the cool mixture was filtered through a medium porousity fritted funnel and rinsed with 2x100 mL of acetone. The solid was collected and allowed to dry to afford the title compound as a white crystalline solid (33.1 g, 118 mmol, 85% yield) which was used without any further purification.

## 4.7 (E)-3-(5-hydroxy-6-methoxy-1H-indol-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one



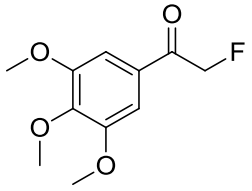

A 2-neck 50 mL RBF was fitted with a magnetic stir bar. The flask was charged with 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.0290 g, 0.100 mmol) and 3,4,5-trimethoxy acetophenone (0.0210 g, 0.100 mmol) in 8 mL of anhydrous 200 proof ethanol. After the reactants were dissolved a catalytic amount of piperidine was added (0.300 mL). The reaction was allowed to stir overnight after which TLC confirmed completion. The solution was then acidified to a pH of 1 with HCl. The reaction mixture was extracted with 2x25 mL DCM. The organic extracts were dried over magnesium sulfate and then concentrated under reduced pressure to afford a brown crystalline solid which was unable to be identified via $^{1}$H NMR.

## 4.8 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone



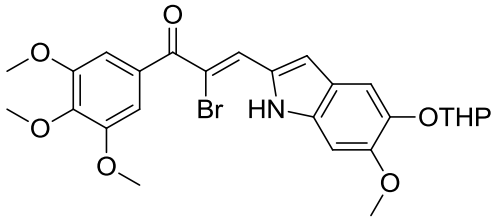

The copper (II) bromide (0.2603 g, 1.17 mmol) and 5 mL anhydrous ethyl acetate were added to an oven dried RBF fitted with a condenser. The green suspension was brought to refluxed at 70˚C. While the reaction mixture was refluxing, a solution of 3,4,5-trimethoxy acetophenone (0.100 g, 0.476 mmol) and 5 mL of anhydrous chloroform were added dropwise. The resulting suspension was allowed to reflux until a color change from green to amber was observed. The solid precipitate was then filtered via gravity filtration and rinsed with ethyl acetate. The remaining solution was dried over sodium sulfate and concentrated under reduced pressure to afford the α-bromo-3,4,5-trimethoxy acetophenone as a brown solid. The product was purified via flash column chromatography to isolate the mono-brominated species. $^{1}$H NMR ($CDCl_3$): δ 7.18 (2H, s), 4.7 (2H, s), 3.86 (9H, s).

## 4.9 2-fluoro-1-(3,4,5-trimethoxyphenyl)ethanone



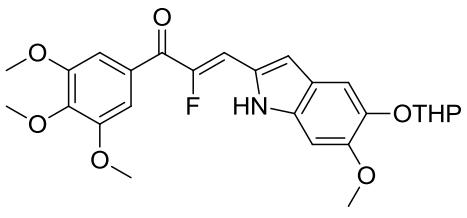

The potassium fluoride (1.61 g, 27.7 mmol) was flamed dried in a two neck RBF fitted with a magnetic stir bar. α-Bromo-3,4,5-trimethoxy acetophenone (0.40 g, 1.38 mmol), 18-crown-6 (1.65 g, 3.22 mmol), and 10 mL anhydrous acetonitrile were added to the flask. The solution was then refluxed for 24 hours at 92 ˚C. After the completion of the reaction was confirmed by TLC, the reaction was concentrated under reduced pressure. The residue was then partitioned between 20 mL of dry DCM and 20 mL of water. The DCM fraction was dried over sodium sulfate. The DCM was then concentrated under reduced pressure to afford the α-fluoro-3,4,5-trimethoxy acetophenone as a tan solid. $^{1}$H NMR ($CDCl_3$): δ 7.20 (1H, s), 5.45 (2H, d), 3.9 (9H, s).

## 4.10 (Z)-2-bromo-3-(6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one



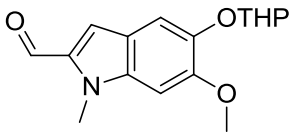

A 2-neck 50 mL RBF was fitted with a magnetic stir bar. The flask was charged with 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.029 g, 0.100 mmol) and 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone (0.021 g, 0.100 mmol) in 8 mL of anhydrous 200 proof ethanol. After the reaction mixture was incorporated a catalytic amound piperidine was added (0.3 mL). The reaction mixture was allowed to stir overnight after which TLC confirmed completion. The reaction mixture was then acidified to a pH of 1 with HCl. The reaction mixture was extracted with 2x25 mL DCM. The organic extracts were dried over magnesium sulfate and then concentrated under reduced pressure to afford a brown crystalline solid which was unable to be identified via NMR.

## 4.11 (Z)-2-fluoro-3-(6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one



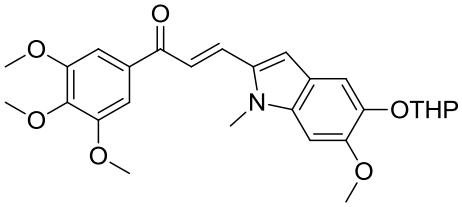
A 2-neck 50 mL RBF was fitted with a magnetic stir bar. The flask was charged with 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.029 g, 0.1 mmol) and 2-fluoro-1-(3,4,5-trimethoxyphenyl)ethanone (0.021 g, 0.1 mmol) in 8 mL of anhydrous 200 proof ethanol. After the reaction mixture was incorporated a catalytic amound piperidine was added (0.3 mL). The reaction mixture was allowed to stir overnight after which TLC confirmed completion. The reaction mixture was then acidified to a pH of 1 with HCl. The reaction mixture was extracted with 2x25 mL DCM. The organic extracts were dried over magnesium sulfate and then concentrated under reduced pressure to afford a yellow crystalline solid which was unable to be identified via NMR.

### 4.12 6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde




A 100 mL RBF was fitted with magnetic stir bar, and rubber septum. The flask was charged with 6 mL of anhydrous DMSO and powdered KOH (0.413 g, 7.36 mmol). The suspension was stirred for 20 minutes and 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.510 g, 1.85 mmol) was added in one portion. Iodomethane (0.546 g, 0.240 mL, 3.85 mmol) was added dropwise after 1 hour to the reaction mixture. After stirring for 2 hours the reaction mixture was poured over 10 mL of ice/cold water, which was placed
in the refrigerator overnight. The resulting suspension was vacuum filtered to afford the product as a brown powder (0.330 g, 62%). $^1$H NMR (400 MHz, $CDCl_3$): δ 9.74 (1H, s), 7.40 (1H, s), 7.12 (1H, s), 6.74 (1H, s), 5.37 (1H, s), 4.07 (3H, s), 3.98 (3H, s), 2.1-1.8 (6H, multiple peaks).

### 4.13 (E)-3-(6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one




A 2-neck 50 mL RBF was fitted with a magnetic stir bar. The flask was charged with 6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.165 g, 0.570 mmol) and 3,4,5-trimethoxy acetophenone (0.119 g, 0.570 mmol) in 8 mL of methanol. After the reactants were dissolved 12M NaOH was added (0.100 mL, 0.00120 mmol). The reaction was allowed to stir overnight after which TLC confirmed completion. The reaction was poured over ice/cold water and allowed to stir until the ice melted. The solid was then filtered using a coarse fritted funnel to afford a brown solid. $^1$H NMR suggests that the desired product is present but further purification is needed.

## 5. Acknowledgements

The author wishes to express her appreciation to Dr. Herman Holt Jr. for the time he set aside every year to teach her new techniques and guide her thorough the research. This project would not be possible without his guidance and research facilities and opportunities. Additionally, she would like to recognize the current and former Holt group

researchers for their support and guidance in this project. She would also like to thank the University of North Carolina Asheville Chemistry department's faculty and staff for their support, patience and direction throughout her career as an undergraduate. This project was funded by the University of North Carolina Undergraduate Research Program, the Chemistry Scholars National Science Foundation Scholarship in Science, Technology, Engineering and Mathmatics (NSF S-STEM) and the Philip G. Carson Distinguished Chair of Natural Science.

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