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Synthesis of Combretastatin A-4 Analog Bearing Indole-Chalcone Moiety

Haley Enten
Chemistry
The University of North Carolina Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Herman Holt Jr.

Abstract

Drugs that inhibit tubulin polymerization have largely been focused on in the field of cancer research. Combretastatin A-4 (CA-4) binds to the colchicine site of β-tubulin, thus inhibiting tubulin polymerization and inducing eventual tumoral vasculature shutdown. Structural modifications of CA-4 have been researched in order to overcome the solubility and stereochemical barriers the drug poses in its original form. Analogs bearing indole and chalcone moiety have shown increased drug efficacy when these structural modifications are implemented in CA-4 compounds. The focus of this project is to synthesize a CA-4 analog containing an indole derivative and a chalcone core. A halogen is incorporated alpha to the carbonyl within the chalcone core of the CA-4 derivative. Synthesis of the substituted indole aldehyde utilizes Hemetsberger-Knittel methodology. The chalcone of interest is formed in the final step through an aldol condensation reaction between a halogenated acetophenone and the indole derivative. A few steps of the indole synthesis were accomplished, but the desired indole aldehyde was ultimately unable to be obtained. The brominated acetophenone was synthesized in several trials and purified via column chromatography. A fluorination reaction involving the purified brominated acetophenone likely yielded the desired fluorinated acetophenone, based on the spectral data acquired after implementing multiple compound identification techniques. Because the indole synthesis prevented the overall synthetic scheme from moving forward, a commercially available indole aldehyde was reacted with brominated acetophenone to form an α-halo indole-chalcone. Further spectral analysis of the product from this aldol condensation reaction is needed to determine if the expected chalcone product was produced. Future research for this project involves synthesizing halogenated indole-chalcones, in which commercially available indoles will be reacted with halogenated acetophenones in order to determine optimal reaction conditions for obtaining the desired chalcone products.

1. Introduction

1.1 Tubulin Inhibition and Microtubule Development

Disruption in the microtubule development in endothelial cells has been a focus over many years in cancer research, as drugs targeting the polymerization of the protein tubulin have shown promising results in treating cancer.[1] Microtubules are cellular components that are made from the polymerization of tubulin, which is composed of an alpha and beta subunit. Microtubules are essential for cell structure, cell signaling, and play a vital role in the mitosis phase of the cell cycle.[1,2] Disturbances in mitotic cell division lead to cell death and prevent progression in tumor development, providing the rationale for focusing on tubulin inhibiting drugs in the field. Tumoral vasculature requires a supply of oxygen and nutrients to be sustained, and apoptosis in the endothelial cells participating in this process can prevent blood flow to these tumors. Limiting the amount of blood delivered to vasculature results in obstructions to tumor growth formation.[3]

In order for cell division to occur, there are various checkpoints the cell must pass in order to move from one phase to the next during mitosis. Microtubules connect to the kinetochores, or centers, of the DNA-containing chromosomes

within cells. Microtubule structure and orientation dictates the shape of mitotic spindle and its ability to align chromosomes during metaphase of mitosis. Chromosomes must be aligned in a precise way along the metaphase plate in order to pass the metaphase-anaphase checkpoint, thus allowing the mitotic spindle to pull the sister chromatids apart, and eventually allowing cell division to occur. Therefore, aberrations to microtubule structure can result in chromosome misalignment, prevent the cell from passing the checkpoint, induce cell arrest, and ultimately result in apoptosis.[4]

Tubulin-targeting drugs are divided into two classes; microtubule stabilizers, such as taxanes and epothilones, and microtubule destabilizers, such as vinca alkaloids and colchicine. Microtubule stabilizers work by changing the conformation of microtubules to a more stable structure, resulting in over-polymerization of tubulin, and subsequently apoptosis. Microtubule destabilizers affect the conformation and shape of microtubules. Destabilizers can shorten the length of the cylindrical microtubules, impeding the correct alignment of chromosomes during metaphase. Additionally, destabilizers can physically interact with the dimers that compose tubulin, creating a bent shape, and can therefore structurally alter microtubule conformation. Instability in microtubule structure causes disruptions in cellular function and results in cell arrest and cell death.[4]

1.2 Combretastatin A-4 and Colchicine

Combretastatin A-4 (CA-4) is a naturally occurring compound isolated from the bark of the South African willow tree *Combretum caffrum* and has shown strong tubulin inhibition activity.[1,2] CA-4 is composed of a trimethoxy A-ring, and a B-ring that possesses a hydroxyl and methoxy group (see Figure 1). The two heterocyclic rings are joined by a *cis*-double bond. CA-4 has a strong affinity to bind to the colchicine site of β-tubulin, making it an effective tubulin polymerization disrupting agent. However, CA-4 fails as a drug for treating cancer due to its poor solubility *in vivo* and its stereochemical properties. The conformation of CA-4 often changes *in vivo* from the necessary *cis*-double bond to the more stable, but less reactive, *trans* conformation, preventing CA-4 from binding to tubulin.[5] Therefore, syntheses of CA-4 analogs and evaluation of their biological properties has emerged as a focus in the field of cancer research. CA4P, a pro-drug and phosphorylated derivative of CA4, is currently in phase III clinical trials and acts as a vascular disrupting agent.[5,6] This pro-drug is water soluble and is a promising anti-cancer agent, however it has displayed negative cardiovascular side effects in patients.[3] The cardiovascular side effects produced by CA4P have often been observed with the presence of tachycardia, bradycardia, and hypertension in a significant amount of patients. Therefore, it is inconclusive as to whether CA4P is actually producing these negative side-effects or is just enhancing cardiovascular disruptions in predisposed patients.[3]

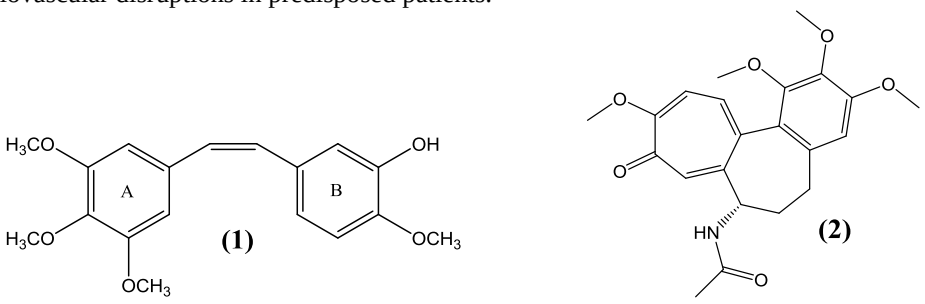


Figure 1. Combretastatin A-4 **(1)** and colchicine **(2)**

As previously stated, drugs that bind to the colchicine site of ß-tubulin are destabilizers and alter the conformation of tubulin. CA-4 binds to colchicine, which then causes the dimers of tubulin to have a flexible conformation. The flexible conformation allows the colchicine-tubulin complex to move inside the cylindrical microtubule, thus obstructing microtubule structure. Colchicine, in its isolated form (Figure 1), is extremely toxic to normal tissues at dosages necessary for tumor disruption, and therefore cannot be used to treat cancer.[4] By binding to colchicine, combretastatins induce vascular shutdown within tumors and leave normal tissues and vasculature intact. CA-4 and its analogs target endothelial cells, which are more abundant in rapidly growing tumors than in normal tissues. Endothelial cells are newly formed cells that have immature cellular structures, and these less developed cellular structures increase sensitivity to microtubule disrupting agents when compared with mature, fully developed cells. Microtubule disrupting agent interaction with endothelial cells leads to cell arrest and blocked blood flow to vascular tumors. Lack of blood flow starves the tumors of oxygen and nutrients, resulting in vascular shutdown within the

tumors.[6] Due to its effectiveness at low dosages and ability to shutdown vascular tumors while leaving normal tissues intact, CA-4 and its derivatives are promising anti-cancer agents.[4,6]

1.3 Structural Alterations to CA-4

In an effort to increase the metabolic solubility and tubulin inhibitory effects of CA-4, changes to structural elements of the compound have been researched. The trimethoxy A-ring is essential for binding activity to the colchicine site of tubulin, based on the loss in potency when these groups are replaced or removed.[3] Therefore, most studies have focused on creating analogs in which changes are made to the *cis*-double bond and the B-ring. A study by Nguyen et al. involved a molecular docking analysis to show the different hydrophobic and hydrophilic interactions between tubulin inhibitory agents and the residues that compose the colchicine binding pocket.[7] The research group concluded that three hydrogen bond acceptors, one hydrogen bond donor, two hydrophobic centers, and one planar group in a compound are needed for optimal binding activity at the colchicine site.[7] Based on structure activity relationships between tubulin inhibitory agents and the colchicine site, a CA-4 analog has been designed in this study as an attempt to satisfy the hydrophobic and hydrophilic interactions required for binding activity.

1.3.1 indoles

Indoles, which are aromatic compounds characterized by a benzene ring fused to a nitrogen-containing pyrrole ring (Figure 2), have also displayed anti-tubulin effects when implemented in heterocyclic ring systems.[8] The tubulin inhibition effects produced by indoles have inspired a number of research groups in the field to incorporate indole moiety when designing CA-4 analogs, in which the B-ring of CA-4 (Figure 1) is replaced by an indole derivative. La Regina et al. conducted a study on heterocyclic compounds bearing indole moiety and a trimethoxy aromatic ring. The tubulin assembly inhibitory effects of the heterocyclic compounds were tested against cancer cell lines.[9] The most active compounds synthesized in the study displayed metabolic stability and solubility, indicating that analogs bearing indole derivatives could improve the efficacy of CA-4.[9] A molecular docking analysis was completed by La Regina et al. and displayed the binding activity of the indole derivatives with the colchicine site. The research group concluded that the trimethoxy groups on one ring formed polar interactions with residues that compose the tubulin binding pocket, and the amine group on the indole ring formed a hydrogen bond with one of the residues.[9] Indoles are found in some naturally occuring tubulin inhibitors, and CA-4 analogs bearing the indole nucleus have been developed to prevent isomerization from the *cis*-double bond to a *trans* conformation, based on these naturally occuring inhibitors.[10] Indoles are highly planar, and could therefore increase stability and prevent isomerization in analogs that incorporate the indole component.

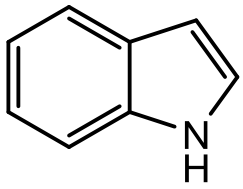


Figure 2. Indole nucleus

1.3.2 chalcones

Chalcones are naturally occuring compounds that have also displayed anti-cancer effects through inducing cell arrest in the mitosis phase of the cell cycle.[8] Chalcones bind to the colchicine site in β-tubulin to inhibit the assembly of microtubules. They are found to be 300 times more potent than the drug colchicine in causing cell arrest, potentiating their ability to act as effective anti-tubulin agents. The way in which chalcones bind to the colchicine site differs from CA-4, suggesting that these compounds belong to different pharmacophore groups.[4] As seen in Figure 3, chalcones are characterized by a carbon chain that contains a carbonyl and a double bond, which joins two aromatic rings. The double bond and carbonyl of the chalcone core has been implemented in various analogs that target tubulin inhibition. It has been concluded that the α,β-unsaturated ketone found in chalcones is necessary for the molecule's functionality.

Additionally, it is noted that the s-*cis* conformation, or *cis* around the sigma bond, is much more active with the cochicine site than the s-*trans* conformation.[11]

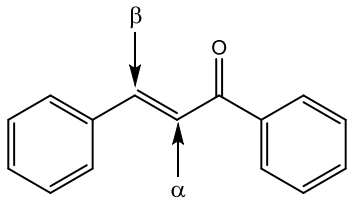


Figure 3. Chalcone structure with α,β-unsaturated ketone labeled

A study completed by Wang et al.[8] focused on the tubulin inhibitory effects produced by indole-chalcone analogs. The analogs synthesized in the study displayed effective cytotoxic results. The most active indole-chalcone was tested against cell lines with resistant phenotypes, and it was concluded that this active compound may be effective in overcoming resistance to anti-tubulin drugs.[8] The results of Wang et al.'s study are important, as easy development of resistance to a drug results in it no longer being efficient, and these results could therefore suggest that heterocyclic compounds bearing indole-chalcone derivatives are promising anti-cancer agents.[8] A study conducted by Ducki et al. on combretastatin-like chalcones yielded results in which the chalcones most resembling CA-4 displayed the most cytoxicity.[6]

1.4 Target Molecule

Based on the promising anti-cancer effects produced by Combretastatin A-4, indoles, and chalcones, a compound consisting of these three molecular components has been designed as an attempt to theoretically increase the tubulin inhibition effects yielded by CA-4 and other anti-tubulin drugs. The target molecule (Figure 4) contains a chalcone core joining the trimethoxy A-ring of CA-4 and an indole derivative. Substituents on the indole ring consist of a hydroxyl and methoxy group at the C5 and C6 positions, respectively. The indole substituents are kept the same as those found on the B-ring of CA-4, in order to further mimic the structural composition of CA-4. Previous work completed by the Holt research group displayed that analog structures retaining the substituents of CA-4, including the three methoxy groups found on the A-ring (see Figure 1), showed the most activity against cancer cell lines.[13] The Holt research group has previously researched and carried out synthetic schemes for acquiring the same target compound, shown in Figure 4, yielding results in which formation of a non-halogenated, indole-chalcone CA-4 analog was achieved through an aldol condensation reaction. However, when utilizing the same reaction conditions that were implemented in the aldol condensation, the α-halo indole-chalcones could not be obtained.[14] Therefore, the aim of the present study was to optimize reaction conditions for synthesizing the halogenated chalcone target molecule (see Figure 4). Another goal of the present research was to build upon and improve prior results relating to the synthesis of the α-halo indole-chalcone. Adding a halogen alpha to the carbonyl could potentially increase binding interactions at the colchicine site of β-tubulin, and could theoretically lock the molecule in the correct conformation (s-*cis*) that is required for binding activity.

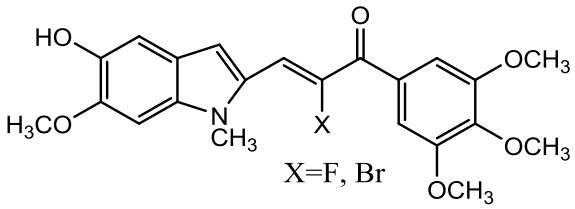


Figure 4. Target Molecule

A study by Wang et al.[8] on indole-chalcone analogs yielded results in which compounds possessing an N-methyl group, as opposed to an N-H group, on the indole nucleus produced more cytotoxic effects (see Figure 5). Wang et al. also determined that increasing the bulkiness of the N group, such as an N-ethyl group (Figure 5), decreased binding

affinity to tubulin due to steric effects. Molecular docking analyses of the methylated indole-chalcones showed that the N-methyl domain of the indole formed hydrophobic interactions with residues at the colchicine site of β-tubulin.[8] A previous study completed by the Holt research group on CA-4 analogs bearing indole-chalcone derivatives also concluded that replacing the amine group with an N-methyl group increased reactivity (see Figure 5).[14] Indoles are highly non-polar, excluding the amine group. The N-methyl group was implemented in the indole moiety of the target compound (Figure 4) in order to increase the non-polar/hydrophobic functionality, and to prevent hydrogen bonding interactions from occurring when synthesis is carried out. As previously stated, a *cis*-conformation of CA-4 is necessary for binding activity at the colchicine site. Figure 3 shows a *trans*-conformation of the chalcone core around the alkene, however, it possesses a *cis*-conformation around the sigma bond. The s-*cis* structure is necessary for chalcone functionality and satisfies the stereochemical requirement for tubulin interaction.[11] The target compound has been designed as an attempt to improve the efficacy of CA-4 as a drug by increasing the hydrophobic and hydrophilic interactions occurring between residues and a tubulin inhibitor at the colchicine site, as well as to improve the solubility properties displayed by CA-4.

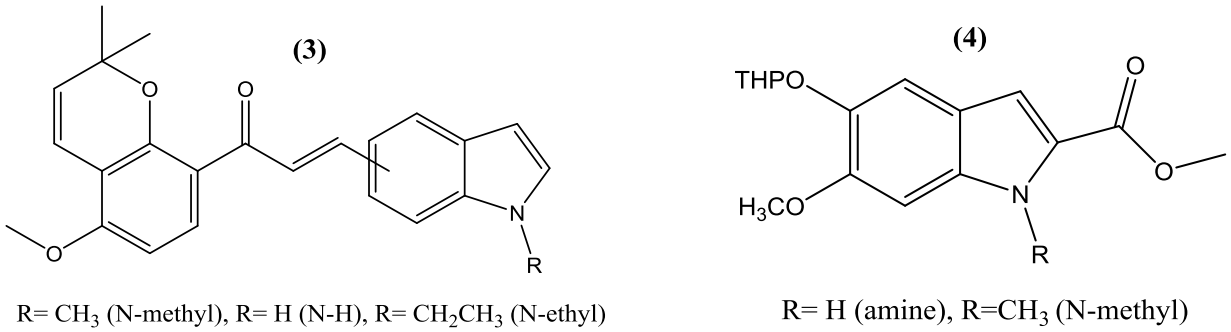


Figure 5. Indole derivatives with varying amino groups assessed by Wang et al.[8] (3) and the Holt research group[14] (4).

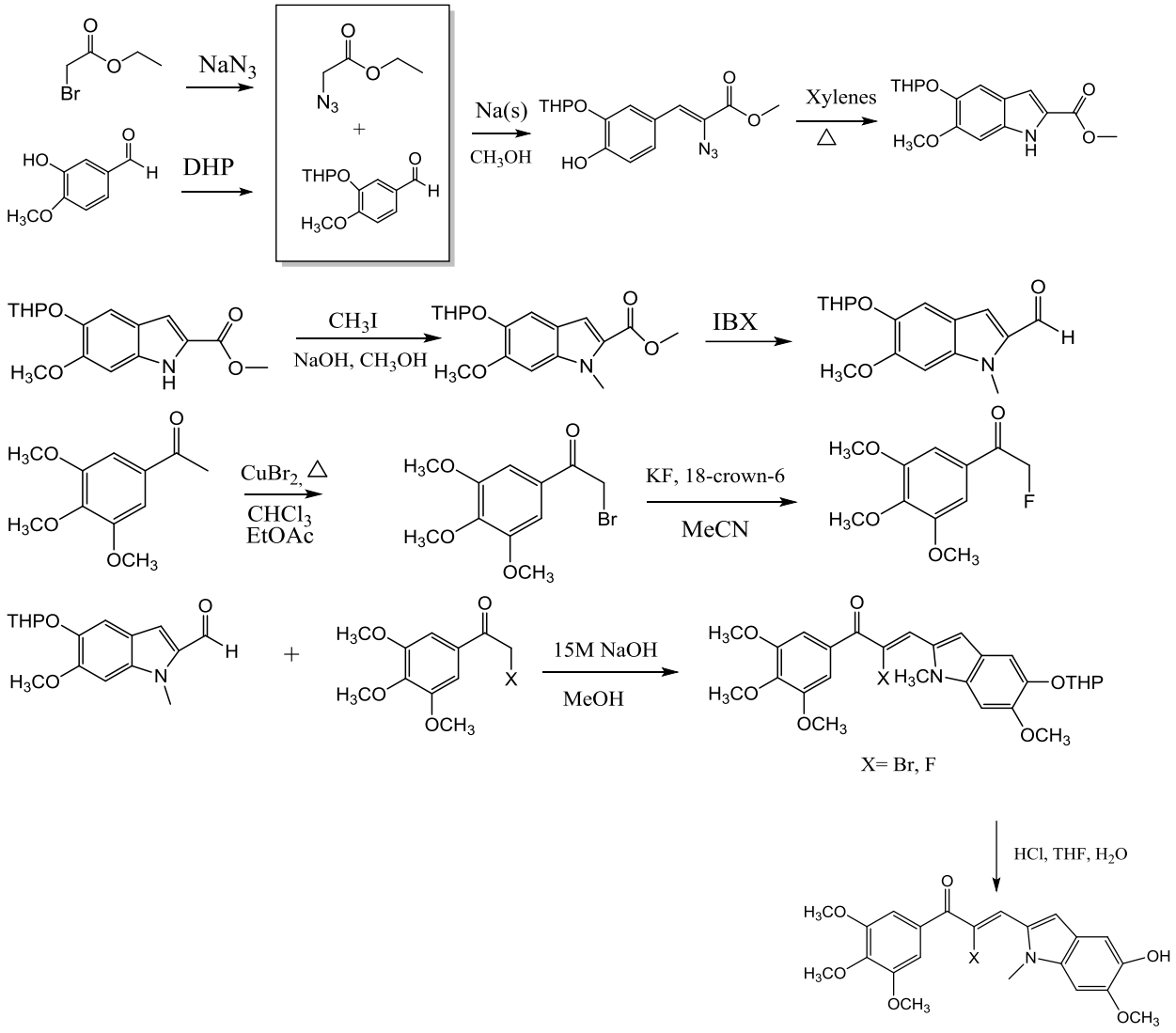
1.5 Goals of Project and Analysis Methods

A target analog of Combretastain A-4 has been designed in the present study in an effort to obtain optimal binding activity at the colchicine site of β-tubulin. The analog has been designed to potentially overcome the solubility and stereochemical barriers that prevent CA-4 from being an effective drug. Previous studies completed by the Holt research group have analyzed pyrazoline derivatives of CA-4, fluorinated CA-4 analogs, indole-chalcone analogs, and analogs where the substituents on the A- and B-rings are altered.[13,14] The goal of the present study was to design a simple synthetic scheme to obtain the target α-halo indole-chalcone molecule (Figure 4) in good yield. The objectives of this study also included determining the effectiveness of the various reactions in the synthetic scheme based on the purity of the compounds synthesized and the resulting percent yields. Optimal reaction conditions for each step of the scheme will be determined, especially focusing on conditions for forming the α-halo chalcones. Identification of intermediate and target compounds in the scheme will be achieved through nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy. Studies on anti-tubulin agents in the field of cancer research often employ further analyses of these agents to test the biological properties and tubulin inhibitory effects produced. Binding activity between the colchicine site and experimental compounds is often assessed through molecular docking and kinetic analyses to determine important binding interactions and affinity of anti-tubulin drugs for the target site. Testing against cancer cell lines is also common in these studies in order to determine *in vitro* tubulin inhibition.[3,5] Further studies investigate the mitotic cellular disruption in mice with cancerous tumors to determine the anti-cancer effects of anti-tubulin agents *in vivo*.[1,8] Based on the available resources for the present study, thin layer chromatography (TLC), ^{1}H-NMR, ^{13}C-NMR, and IR spectroscopy were utilized for identification of the target compound and intermediate compounds in the synthetic scheme. The experimental methods for synthesizing the substituted indoles and intermediate compounds were based on work done by previous students in this research group, and alterations to the methods were developed as the reactions were carried out. Hemetsberger-Knittel methodology is utilized to synthesize the substituted indole. The chalcone of interest (Figure 4) can then be formed through an aldol condensation reaction with varying reaction conditions.

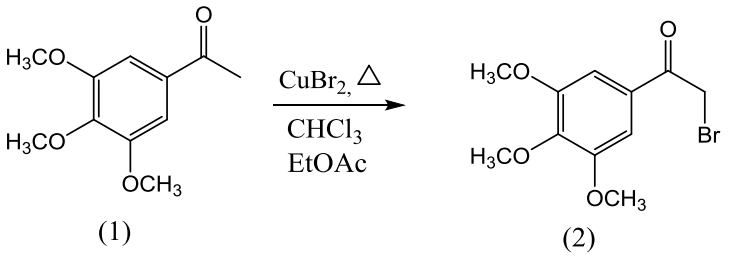
2. Results and Discussion

The target molecule (Figure 4) can be synthesized through an aldol condensation reaction involving the halogenated acetophenones and the substituted indole aldehyde. The starting material for the halogenated acetophenones, which is 3,4,5-trimethoxy acetophenone, can be purchased from Sigma Aldrich. The substituted indole can be synthesized initially from ethyl 2-bromoacetate and 3-hydroxy-4-methoxybenzaldehyde (Scheme 1), which are also commercially available starting materials. A protecting group is first added to the hydroxyl group of the benzaldehyde before it is incorporated into the indole synthesis, in order to prevent any hydrogen bonding interactions. Thermolysis of the vinyl azide allows the indole ring to form, and the indole ester can then be reduced and selectively oxidized to obtain the desired indole aldehyde. The indole aldehyde can then be reacted with an α-halo acteophenone to yield the target chalcone through an aldol condensation.

Scheme 1 (shown below). Synthetic Scheme to Obtain Target Indole-Chalcone Analog



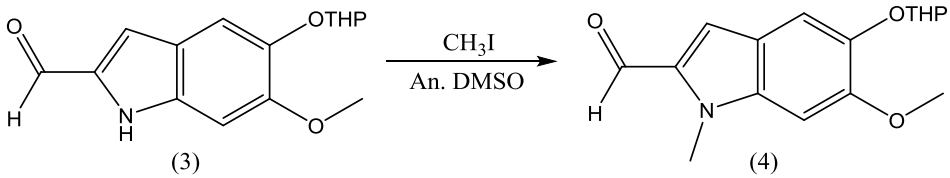
Scheme 2 (shown below). Brominated acetophenone



(1) (2)

The reaction to synthesize α-bromo-3,4,5-trimethoxy acetophenone (Scheme 2, compound 2) from 3,4,5-trimethoxy acetophenone and copper (II) bromide resulted in varying overall yields ranging from 19% to 58%. The products from each trial were either a dark brown solid or a dark brown oil after concentrating under reduced pressure. The molar equivalents for each trial were kept the same, in which the equivalents of $CuBr_2$ were 1.5 to the trimethoxy acetophenone. Scaling up the reaction starting material did not consistently improve yields. Reaction times were increased from an hour to an hour and 45 minutes, as TLC and ^{1}H-NMR showed starting material in the initial trials. Increasing the reaction times caused initial formation of the di-α-brominated product, however the amounts formed were miniscule relative to the amounts of mono-α-brominated product and starting material present at the end of each reaction trial. All of the products from the 4 trials possessed starting material, and were therefore combined and purified via column chromatography to obtain the purified α-bromo-3,4,5-trimethoxy acetophenone. Reaction times and the $CuBr_2$ molar equivalent will be increased in future trials to reduce the amount of starting material present in the desired product.

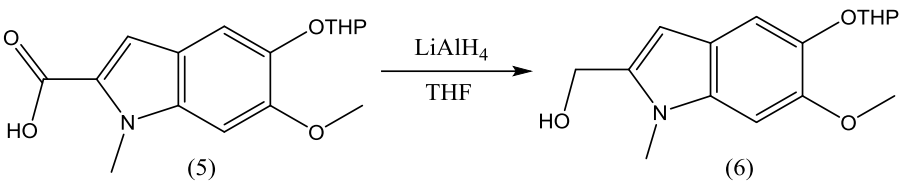
Scheme 3. Methylation of Substituted Indole



(3) (4)

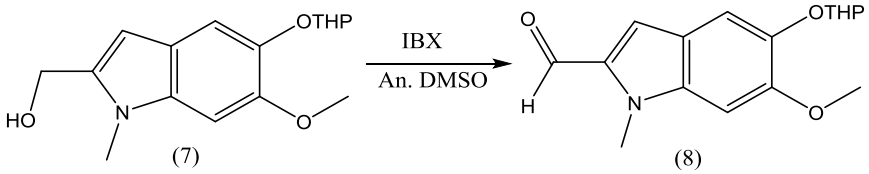
6-Methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (Scheme 2, compound 3) had been synthesized by a previous student, and was used to methylate the nitrogen in this substituted indole. The previously synthesized indole was used as an attempt to skip some steps of the synthetic scheme so that reactions involving the α-halo chalcone target molecules could be focused on. Methylation of the indole hydrogen is necessary to prevent the hydrogen from reacting in the future chalcone synthesis step. Prior to the methylation reaction, ^{1}H-NMR showed that the previous student's substituted indole compound was pure, and possessed a characteristic aldehyde peak around 9.8 ppm, as well as an N-H peak around 8.65 ppm. Methylation was carried out through reacting the substituted indole (3) with iodomethane to yield light brown crystals. ^{1}H-NMR results confirmed that the indole hydrogen had been methylated through the disappearance of the N-H peak, and the appearance of an additional peak in the methyl region of the NMR spectrum. However, the compound no longer possessed the aldehyde functional group, and the product was thought to have a carboxylic acid or ester instead. Perhaps the aldehyde functionality was lost because the aldehyde peak produced by the initial substituted indole (3) was small, and did not show up after re-doing ^{1}H-NMR analysis of this indole starting material. IR analysis of the indole starting material and indole product (4) also did not verify whether the functional group was a carboxylic acid or ester in either compound. Therefore, a reduction of the proposed carboxylic acid product was completed in the next reaction so that it could then be selectively oxidized to the desired aldehyde.

Scheme 4. Reduction of carboxylic acid



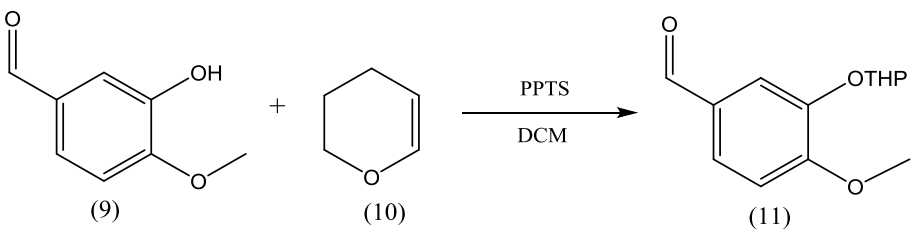
Reduction of the speculated carboxylic acid product (5) to a primary alcohol (6) was attempted by reacting the proposed 6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylic acid with $LiAlH_4$ (Scheme 4). TLC did not verify reaction completeness after stirring overnight, however the reaction workup was carried out anyways. Concentrating under pressure resulted in a light orange solid product. ^{1}H-NMR analysis showed that the carboxylic acid was reduced to either the desired primary alcohol or a methyl group. An attempt was made in the next reaction to obtain the desired aldehyde through oxidation of the inconclusive alcohol or methyl group.

Scheme 5. Oxidation of primary alcohol or methyl to desired aldehyde



Oxidative reaction conditions were used in order to synthesize 6-methoxy-1,2-dimethyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole (Scheme 5, compound 8). Oxidation of the speculated alcohol or methyl group to yield the desired aldehyde was carried out by reacting the substituted indole (7) with IBX (Scheme 5). After stirring overnight, TLC only showed one spot for the product. IBX did not show up on TLC because it had most likely reached its full oxidative potential. The flask containing the substituted indole product from the previous reaction was not saved, so reaction completeness could not be confirmed through TLC analysis. Despite inconclusive TLC analysis, the reaction mixture was washed, followed by concentrating under pressure to afford light yellow crystals. ^{1}H-NMR data still did not show that the desired indole-aldehyde had been isolated, as the characteristic aldehyde signal that typically occurs between 9-10 ppm was not present in the spectrum. Therefore the product was not used in the rest of the reaction scheme.

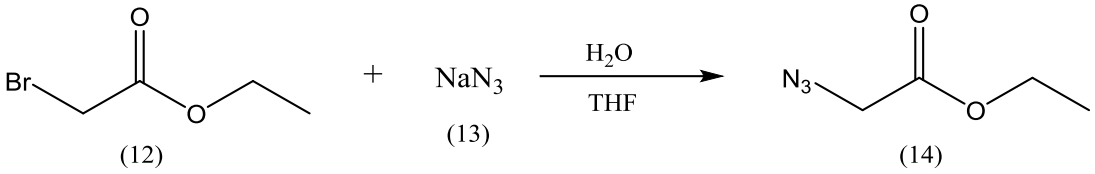
Scheme 6. Protection of alcohol using DHP



The synthesis of the target indole derivative using a previous student's substituted indole compound was unsuccessful, so indole synthesis was started from the beginning with commercially available starting material. 4-Methoxy-3-

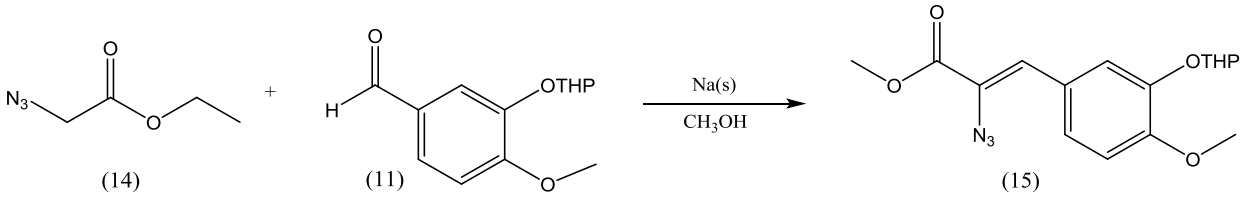
((tetrahydro-2H-pyran-2-yl)oxy) benzaldehyde (11) was synthesized from 3-hydroxy-4-methoxybenzaldehyde (9) and 3,4-dihydro-2H-pyran (10) to afford a light-orange oil (Scheme 6). Adding the protecting group to the alcohol was a necessary step to prevent any interactions the alcohol might have with reagents in the rest of the synthetic scheme. The protected benzaldehyde was formed with a yield of 79%. ^{1}H-NMR analysis confirmed that the protecting group had been added and the desired product was obtained. The THP group was characterized by a triplet peak (1H) occurring at 5.44 ppm, as well as coupled multiplets occuring in the regions of 3.66-3.41 ppm (2H) and 2.06-1.49 ppm (6H).

Scheme 7. Synthesis of ethyl azidoacetate



Ethyl azidoacetate (Scheme 7, compound 14) was synthesized from ethyl bromoacetate (12) and sodium azide (13). Two trials in which methanol was used as the solvent yielded products that had a mixture of starting material and ethyl azidoacetate. Therefore, tetrahydrofuran (THF) was used as the solvent in the third trial, which afforded the pure ethyl azidoacetate product as a light orange oil, with a yield of 74%. TLC showed slight separation between spots for starting material and product, and ^{1}H-NMR analysis confirmed that the desired product had been formed. The success of the third trial could be due to increased miscibility of ethyl bromoacetate in THF, as opposed to in MeOH, resulting in optimized reaction conditions.

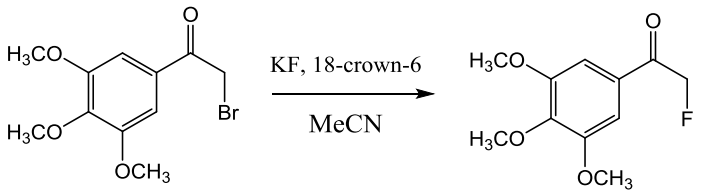
Scheme 8. Formation of vinyl azide



Forming the vinyl azide moiety in the desired product, (Z)-((2-azido-3-(4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)acryloyl)oxy)methylium (see Scheme 8, compound 15), was attempted through an aldol condensation reaction involving ethyl azidoacetate (14) and 4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy) benzaldehyde (11) as starting material (Scheme 8). This reaction precedes indole ring formation, as thermolysis of the vinyl azide can allow the indole product to be acquired. Before the ethyl azidoacetate product produced by the reaction shown in Scheme 7 was acquired, pure ethyl azidoacetate synthesized by another research student was reacted with the protected benzaldehyde (11) using reaction conditions involving sodium metal and methanol. Two trials were completed using the reagents and reaction conditions just stated, but proved to be unsuccessful. The ^{1}H-NMR spectra of the products yielded from the two trials possessed a strong signal occurring around 9.8 ppm, a signal characteristic of aldehydes. It was determined that the two products from these trials were mainly composed of the protected benzaldehyde (11). Therefore, a new method for obtaining the vinyl azide product was trialed. Fresh ethyl azidoacetate was synthesized (Scheme 7) and reacted with protected benzaldehyde (11), in which the new reaction conditions involved potassium tert-butoxide and THF. The reaction yielded similar results to those of the first two trials, as protected benzaldehyde was the major product observed after running the reaction. The vinyl azide reaction (Scheme 8) was carried out one more time, in which the freshly made ethyl azidoacetate and the original sodium metal/methanol reaction conditions were trialed. The fourth trial gave the undesirable result of the protected benzaldehyde being the main product of the reaction, once again. After several attempts, it was clear that the vinyl azide reaction was preventing the indole

synthesis from moving forward. Therefore, synthesis shifted in a new direction, in which the α-halo indole chalcones would try to be synthesized from commercially available indole starting material, thus allowing the indole synthesis to be circumvented.

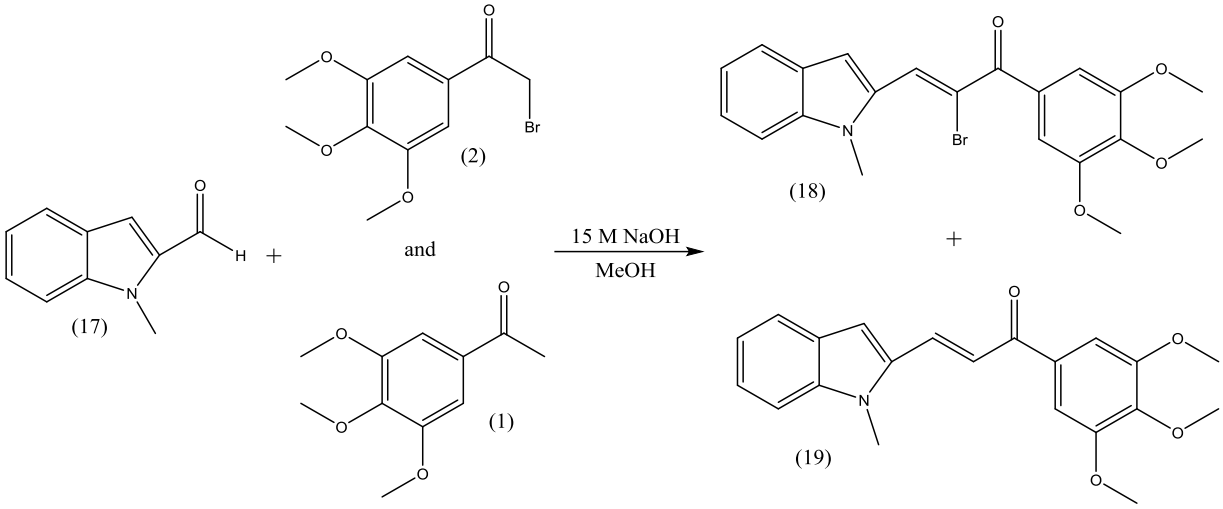
Scheme 9. Fluorinated acetophenone



(1) (16)

Synthesis of 2-fluoro-1-(3,4,5-trimethoxyphenyl)ethanone (see Scheme 9, compound 16) was carried out using the purified, brominated acetophenone product (2) acquired from the reaction outlined in Scheme 2. The crown ether, 18-crown-6, acted as a chelator to the potassium ion of potassium fluoride, allowing for more efficient substitution of the fluoride ion for the bromine found in compound (1). It must be noted that the author used a 20 molar equivalent of potassium fluoride to the brominated acetophenone, which was based on a prior research student's procedure, but this 20 molar equivalent is extremely excessive and could be reduced to a 3 molar equivalent. After refluxing the reagents in acetonitrile for 24 hours, TLC showed that the reaction had gone to completion. Upon ^{1}H-NMR analysis, the product of the reaction produced a probable doublet peak occurring at 7.19 ppm, which was believed to be representative of the fluorine in the desired product. Fluorine is known to undergo spin coupling with neighboring protons, therefore producing a doublet peak instead of what would would otherwise be a singlet. However, this signal was of low intensity and further identification needed to be completed. IR analysis of the product did not further elucidate whether the fluorinated acetophenone had been obtained. Signals produced by C-F stretches occur at frequencies in the region between 1100 to 1400 cm^{-1} of the IR spectrum, and are expected to absorb strongly in this region. The C-F stretch could not be distinguished from other signals in the spectrum, as C-O stretches from the three methoxy groups of acetophenone also strongly absorb in this region of 1100 to 1400 cm^{-1}. Therefore, Liquid chromatography-mass spectrometry (LCMS) was utilized to gain further information about the identity of the product. The major peak seen in the LCMS spectrum had a mass to charge ratio representative of trimethoxy acetophenone alone. This could have been due to the fluoride ion leaving the acetophenone structure, after being ionized by the instrument. A signal representative of the total mass of the fluorinated acetophenone was not apparent in the spectrum. However, after re-examining the proton NMR data produced by the product, the presence of trimethoxy acetophenone was ruled out. Additionally, it was not expected that the trimethoxy acetophenone would be present in the product, as the purified brominated acetophenone compound was used as starting material for the reaction. Therefore, it can be said with confidence that the desired fluorinated acetophenone product was produced from the reaction.

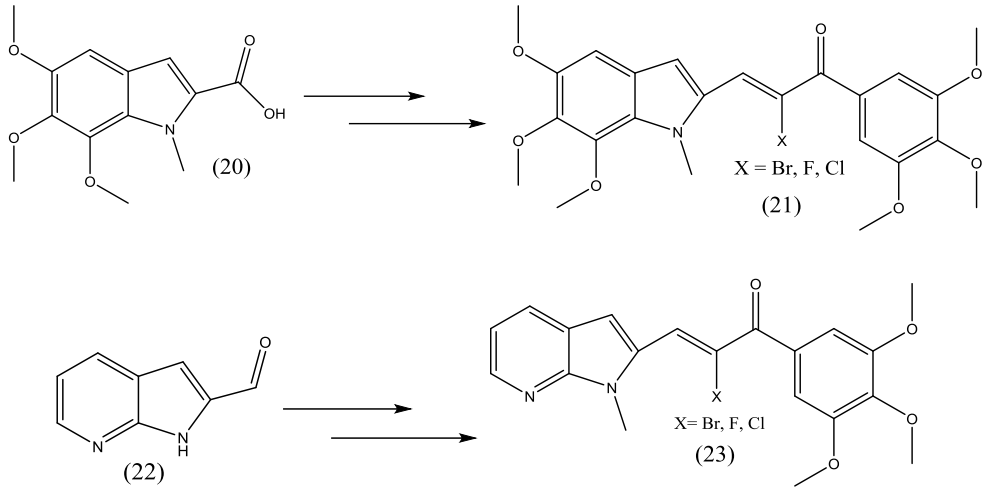
Scheme 10. α-halo indole-chalcone



Since the substituted indole aldehyde product (8) could not be obtained using Hemetsberger-Knittel methodology, a commercially available indole, 1-Methylindole-2-carboxaldehyde, was used to surpass the indole synthesis portion of the target molecule synthetic scheme (Scheme 1). The lack of substituents on the methylindole carboxaldehyde would likely decrease the affinity of the compound for the colchicine site of β-tubulin, thus lowering its tubulin inhibition effects. However, for the purposes of this undergraduate research project, using an un-substituted indole derivate as starting material for forming the chalcone product allows the optimal reaction conditions of the chalcone synthesis to be determined. All of the purified brominated acetophenone obtained via column chromatography (see Results, Scheme 2) had been used to carry out the fluorination reaction outlined in Scheme 9. An α-bromo trimethoxy acetophenone (2) and trimethoxy acetophenone (1) mixture was left over from a previouis reaction that was completed (Scheme 2), and was therefore used as starting material in the present chalcone reaction. It was believed that separation of the two expected chalcone products, (18) and (19), via column chromatography would be less difficult than separation of the brominated acetophenone (2) and acetophenone (1) mixture. Two prior chalcone reactions, in which 3,4,5-trimethoxy acetophenone and 3,4,5-trimethoxy benzaldehyde were used as starting material, had been carried out to determine optimal reaction conditions for the present aldol condensation reaction. Reaction conditions outlined by Ducki et al.[6] involve 50% (w/v) $NaOH_{(aq)}$ and MeOH, followed by acidification with HCl after stirring overnight. The Ducki conditons did not yield the desired chalcone product. When 15 M $NaOH_{(aq)}$ and MeOH (without acidification) were trialed, the desired chalcone product was obtained in high yield.

Therefore, 15M $NaOH_{(aq)}$ and MeOH were used for the present reaction (Scheme 10). Upon completion of the aldol condensation (Scheme 10), TLC showed what appeared to be the presence of two products in the dark red-orange solution, as well as the indole starting material. Vacuum filtration afforded the product as an orange-crystalline solid. ^{1}H-NMR analysis did not verify which chalcone product was obtained. The non-halogenated chalcone product (see Scheme 10, compound 19) was ruled out as only one alkene signal, representative of a chalcone, appeared in the NMR spectrum and occurred at 7.50 ppm. Multiple signals occurring in the methoxy and methyl region of the NMR spectrum indicated the presence of two chalcone products. However, fewer signals occurring in the aromatic region, as well as the region where the chalcone signal would occur, of the spectrum was contradictory to the number of signals occurring in the methoxy and methyl regions. The 15 M NaOH could have potentially reacted in excess, resulting in a chalcone product where the bromine of (18) is replaced with an alcohol group. Further analysis and identification of the product of this reaction is needed before any conclusions can be drawn about which chalcone product was formed, or if a chalcone product formed at all.

Scheme 11. Opportunities for future research students involving indole-chalcones



Various problems arose when attempting the indole synthesis using Hemetsberger-Knittel methodology, and prevented future synthetic steps towards obtaining an indole-chalcone CA-4 analog from being completed. In order to circumvent the indole synthesis, a comercially available indole (17) was implemented in Scheme 10 to carry out the aldol condensation reaction for synthesizing chalcones. Previous students in the Holt research group have successfully completed the indole synthesis and acquired the desired indole aldehyde (8), but were unable to synthesize the final chalcone target molecule (Figure 1).[14] Therefore, a focus on synthesizing halogenated indole-chalcones is needed for the research project to move forward. Reacting commercially available indoles or pyrrolo pyridines with halogenated acetophenones allows for a simple synthetic scheme to be utilized for obtaining the desired chalcone products. A more direct synthetic route is accomplished when implementing commercially available indoles, as opposed to first synthesizing the substituted indole aldehyde (8) prior to completing the chalcone reaction.

Example reactions involving commercially available indoles and pyrrolo pyridines that could be trialed by future research students are given in Scheme 11. If the indole compound 5,6,7-trimethoxy-1-methyl-1*H*-indole-2-carboxylic acid is utilized in the chalcone synthetic scheme, reduction of the carboxylic acid to a primary alcohol would first need to be completed. Oxidation of the alcohol to the desired indole aldehyde would then allow the indole product to be reacted with a halogenated acetophenone, so that the desired chalcone (21) could be produced. The reaction involving the pyrrolo pyridine (22), shown in Scheme 11, to obtain the desired chalcone (23) also involves a couple of intermediate steps. Methylation of 1*H*-pyrrolo[2,3-*b*]pyridine-2-carbaldehyde is followed by reacting this methylated product with a halogenated acetophenone to give the chalcone product (23). Reactions involving the commercially available starting material, such as the indole (20) and pyrrolo pyridine (22) shown in Scheme 11, allows the focus of the research project to involve optimizing reaction conditions and determining the best reagents to use for synthesizing α-halo indole-chalcones.

3. Conclusion

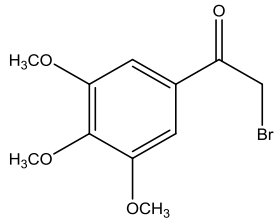
The goal of this research project was to synthesize the target molecule (Figure 4), which was designed to implement structural components of indoles, chalcones, and Combretastatin A-4, based on the promising anti-cancer effects produced by these three compounds. Bromination of acetophenone (Scheme 2) required several trials, as the reactions yielded products in which a mixture of the trimethoxy acetophenone starting material and the brominated product was produced. The fluorinated acetophenone (16) was likely obtained in one trial, based on the spectroscopy data obtained from performing various identification techniques. The fluorinated product (16) still needs to be reacted with an indole aldehyde in order for the fluorinated indole chalcone to be produced. Synthesis of the desired indole aldehyde

derivative using Hemetsberger-Knittel methodology proved to be difficult, with several reaction trials having to be performed for many steps of the indole synthetic scheme. Therefore, commercially available indole and pyrrolo pyridine derivatives will be reacted with halogenated acetophenones to obtain a variety of α-halo chalcones that bear indole moiety. Comercially available indoles and pyrrolo pyridines allow the indole synthesis to be avoided, and allow for a more direct route in synthesizing the desired chalcone products. Optimizing reaction conditions and designing synthetic schemes for obtaining these halogenated indole-chalcone CA-4 analogs could be a plausible research project for future students in the Holt research group.

4. Experimental

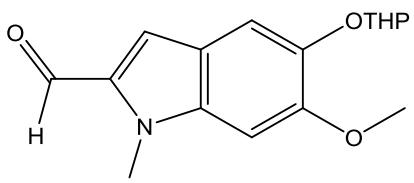
Deuterated chloroform ($CDCl_3$) was used as a solvent to prepare all NMR samples. The NMR spectra were obtained using a Varian Unity INOVA instrument with an Oxford Instruments 400 MHz superconducting magnet. Infrared (IR) spectra were obtained using a Thermo-Fischer Scientific Nicolet iS10 FT-IR spectrometer. The reagents for all reactions were commercially bought. The solvents for water-sensitive reactions were dried over 3Å molecular sieves, and were allowed to sit overnight.

4.1 *2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone*



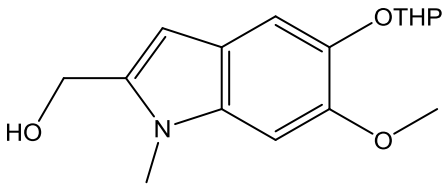
An oven dried RBF was fitted with a condenser and put under a stream of nitrogen. Added to the flask was 2.00 g (9.51 mmol) of 3',4',5'-trimethoxy acetophenone and 5 mL of anhydrous chloroform. In a separate oven dried RBF, 3.19 g (0.0143 mol) of copper(II) bromide and 5 mL of anhydrous ethyl acetate were combined under inert conditons. The $CuBr_2$ and ethyl acetate solution was brought to reflux at 70 °C. Once the green solution reached 70 °C, the acetophenone and chloroform solution was added to the $CuBr_2$ solution via syringe. Although TLC showed that starting material was still present, the reaction was stopped after an hour and 45 minutes to prevent the di-brominated product from forming. The solid precipitate was filtered from the desired solution using gravity filtration, and was rinsed with ethyl acetate. The solution was then dried with sodium sulfate and was concentrated under reduced pressure to afford a brown oil. The products from the 4 trials all contained starting material. Therefore, the products were combined and purified through column chromatography using a Biotage flash column.
^{1}H-NMR ($CDCl_3$): δ 7.26 (2H, s), 4.43 (2H, s), 3.95 (3H, s), 3.94 (6H, s).
IR: 1681 cm^{-1} (C=O stretch), 1580 cm^{-1} (C=C aromatic stretch), 1328 cm^{-1} (C-H bend), 1215 cm^{-1}, 1124 cm^{-1}(C-O stretch), 1000 cm^{-1}.

4.2 *6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde*



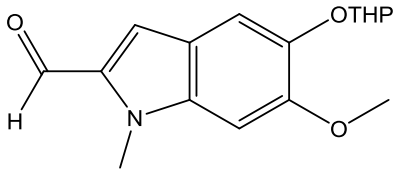
A 100 mL RBF was oven-dried, fitted with a stir bar, and put under N_2 gas. Added to the RBF was 6 mL of anhydrous DMSO and 0.422 g (7.52 mmol) of powdered KOH. After the mixture was allowed to stir for 20 minutes, 0.510 g (1.85 mmol) of 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde, which had been synthesized by a previous student, were added to the flask. After the indole/KOH/DMSO mixture had stirred for one hour, 0.240 mL (0.546 g, 3.85 mmol) of iodomethane were added to the flask via syringe. TLC verified reaction completeness 1 hour after the iodomethane was added to the reaction vessel. The reaction mixture was then poured over ice-cold water in a 100 mL RBF, and the solution turned from medium brown to light brown. The flask was stored in the refrigerator overnight, and the light brown crystals were filtered on a fritted funnel and rinsed with water the next day. Methylation of the substituted indole yielded 0.622 g of product, in which methylation was successful but the compound no longer possessed an aldehyde group based on ^{1}H-NMR and IR spectra.

4.3 *6-methoxy-1,2-dimethyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole*



A 2-neck 100 mL RBF was oven-dried, fitted with a stir bar, and put under a stream of nitrogen. In the RBF, 0.330 g (8.70 mmol) of $LiAlH_4$ and 10.0 mL anhydrous THF were combined, and the flask was then put in an ice bath. Added to a separate oven-dried flask with stirring were 0.55 g of the speculated 6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylic acid and 5.0 mL anhydrous THF. The indole solution was added to the $LiAlH_4$/THF mixture via syringe, and the reaction was allowed to stir overnight. TLC did not verify reaction completeness after stirring overnight, however the reaction workup was continued anyways. The reaction mixture was diluted with 20.0 mL diethyl ether and 3.0 mL saturated NH_4Cl via syringe. No vigorous reacting was observed. The solution was filtered through a fritted funnel and was then washed with 2 x 20 mL of diethyl ether. The product was then concentrated under reduced pressure and put on the high vaccum to afford a light orange solid (0.40 g). ^{1}H-NMR data showed that the carboxylic acid had either been reduced to a primary alcohol or methyl.

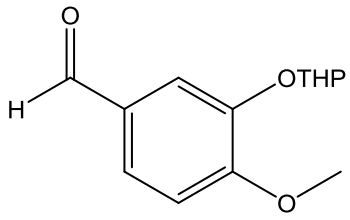
4.4 *6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde* (from oxidation)



A 100 mL RBF was fitted with a stir bar and put under a stream of nitrogen. About 0.4 g of the speculated (6-methoxy-1-methyl-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl) methanol product from the previous reaction were added to the RBF. After adding the indole derivative, 50 mL of anhydrous DMSO were added to the flask with stirring. Finally, 0.846 g (3.01 mmol) of IBX were added to the RBF, and the reaction mixture was allowed to stir overnight. TLC did not verify reaction completeness, but the reaction had been stirring overnight so the product was diluted with 60 mL ethyl acetate and 60 mL saturated $NaHCO_3$. The solution turned from a dark orange to a murky green-brown color. The mixture was vaccum filtered through a fritted funnel with a pad of celite to remove excess IBX. The filter was rinsed with 60 mL of ethyl acetate, and the reaction mixture was then extracted with 2 x 30 mL ethyl acetate. The combined organic layers were washed with brine and dried with Na_2SO_4. Concentrating under reduced pressure

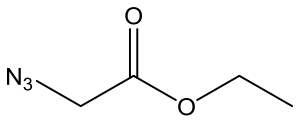
yielded light yellow crystals (0.20 g). The desired aldehyde product was not isolated based on ^{1}H-NMR and IR analysis, and the product was proposed to possess an ether group instead.

4.5 *4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)benzaldehyde*



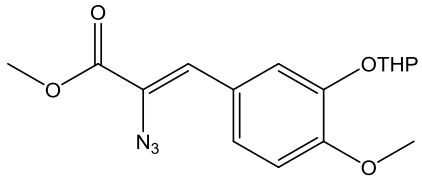
A 2-neck 250 mL RBF was oven dried and put under a stream of nitrogen. After the flask had cooled, 3.221 g (21.2 mmol) of 3-hydroxy-4-methoxybenzaldehyde and 100 mL anhydrous DCM were added to the flask with stirring. In the same flask, 0.795 g (3.18 mmol) of pyridinium p-toluene sulfonate (PPTS) were then added, and the mixture was stirred until complete dissolution. After the PPTS had dissolved, 11.9 mL of 3,4-Dihydro-2H-pyran (DHP) were added to the RBF. About 2 hours later another 0.2 mL of DHP were added to the flask and the reaction was allowed to stir overnight. The following day TLC confirmed the formation of a product. The reaction mixture was concentrated under reduced pressure to remove excess DCM. The oil was transferred to a separatory funnel with 100 mL diethyl ether. Three layers formed, indicating that DCM was still present in the mixture. The mixture was then emptied into a beaker, dried with Na_2SO_4, and put on the rotovap to give the remaining oil. The oil was washed with 100 mL diethyl ether. Only one layer formed and was therefore assumed to be all organic. A 200 mL saturated solution of K_2CO_3 was made for further extraction. The reaction mixture was initially washed with 75 mL of the K_2CO_3 solution, causing the mixture to turn yellow. The reaction mixture was washed 2 more times with the saturated solution, and the combined organic layers were then dried with Na_2SO_4. The remaining solution was concentrated under reduced pressure and put on the high vaccum to afford a light orange oil (3.93 g). ^{1}H-NMR ($CDCl_3$): δ 9.84 (1H, s), 7.64 [1H, d (from long-range coupling)], 7.54 (1H, d), 7.52 (d from coupling), 7.01 (1H, d), 5.44 (1H, t), 3.94 (3H, s), 3.66-3.41 [2H, m (with coupling)], 2.06-1.49 [6H, m (with coupling)].

4.6 *ethyl azidoacetate*



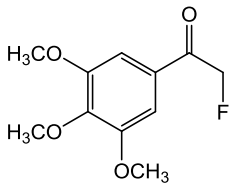
A 3-neck 100 mL RBF was fitted with a condensor, thermometer, and stir bar. Sodium azide (15.11 g) and 26 mL of H_2O were added to the flask and allowed to stir. THF (30 mL) and 17.2 mL of ethyl bromoacetate were added to the reaction flask, and the mixture was heated to 70°C. The reaction mixture was allowed to stir overnight at this temperature, and had turned a pale tan color overnight. TLC did not confirm reaction completeness, however a pale tan color of the solution indicates progress of the reaction. After cooling to room temperature, the reaction mixture was poured over 60 mL of H_2O and washed with 3 x 40 mL of diethyl ether. The solution was then dried over Na_2SO_4. Concentrating under reduced pressure afforded a light orange oil.
^{1}H-NMR ($CDCl_3$): δ 4.24 (2H, m), 3.82 (2H, s), 1.28 (3H, t).

4.7*(Z)-((2-azido-3-(4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)acryloyl)oxy) methylium*



A 100 mL oven dried RBF was fitted with a condensor and stir bar, and was then put under a stream of nitrogen. After the flask cooled, 5.00 mL of dry MeOH were added to the flask and was submerged in an ice bath. About 0.3 grams of sodium metal were added to the flask with stirring, and the Na(s) was allowed to dissolve. A separate flask containing the protected benzaldehyde product (0.82 g) from a previous reaction was put on a vaccum to remove as much air as possible from the flask. Ethyl azidoacetate (1.11 g) was weighed into the flask containing the protected benzaldehyde, and was then put under a stream of nitrogen. Dry methanol (4.00 mL) was added to the ethyl azidoacetate/protected benzaldehyde mixture with stirring. After the Na (s) had dissolved, the flask was submerged in a methanol/dry ice bath to get the mixture to -10° C. The dark orange ethyl azidoacetate/protected benzaldehyde mixture was added to the reaction vessel via syringe, and the reaction mixture was initially a light orange slurry. The slurry got darker over time, marking progress of the reaction, and was allowed to stir in the dry ice bath for 4 hours. After 4 hours, the reaction mixture was allowed to gradually warm to room temperature and stir overnight. The next day, TLC showed that the reaction mixture was likely still the protected benzaldehyde, but further reaction steps were continued anyways. The yellow-orange slurry was poured over crushed ice and saturated NH_4Cl, and was then washed with 2 x 40 mL diethyl ether. The solution was dried over Na_2SO_4 and concentrated under reduced pressure to give an orange-brown oil. ^{1}H-NMR analysis showed that the product was mostly protected benzaldehyde.

4.8 *2-fluoro-1-(3,4,5-trimethoxyphenyl)ethanone*



A 3-neck 100 mL RBF was oven dried and fitted with a thermometer adapter and condensor. Potassium fluoride (0.92 g, 15.9 mmol) was added and flame dried in the flask. The vessel was cooled under a stream of nitrogen, and 0.5 g of 18-crown-6-ether and 8 mL of anhydrous acetonitrile were added to the flask with stirring. The purified brominated acetophenone (0.23 g, 0.795 mmol) was dissolved in 2 mL anhydrous acetonitrile and was quickly added to the reaction flask. The solution was heated to 80°C, but a few hours later the majority of the solvent had boiled off. Therefore the heat was turned down and 15 more mL of acetonitrile were added to the flask. The solution was supposed to reflux for 24 hours, but after stirring overnight, the solvent had evaporated off and a black solid remained. More acetonitrile was added to the flask (15 mL), and TLC of the reaction mixture portrayed that the reaction had gone to completeness. The reaction mixture was allowed to cool and was then transferred to a 100 mL RBF. Concentrating under reduced pressure gave a dark brown solid. The mixture was washed with 35 mL DCM and 30 mL of H_2O. Three layers formed in the separatory funnel, so 25 mL diethyl ether was added to the solution. It was then observed that a brown precipitate had formed, and was filtered from the solution so that the contaminant could be removed. Adding 15 more mL of diethyl ether resulted in the formation of two liquid layers in the separatory funnel, and the organic and aqueous layers were then able to be separated. The combined organic layers were dried over Na_2SO_4, and concentrating under reduced pressure afforded the product as a brown-tan solid.

4.9 *α-bromo indole-chalcone and indole-chalcone*

A 100 mL RBF was flame dried and fitted with a condensor, and put under N_2(g) (although the reaction uses water, inert conditions were used as this afforded a chalcone product in high yield in a trial reaction). MeOH anhydrous (30 mL) and 0.88 g of 1-Methylindole-2-carboxaldehyde were added to the flask with stirring. The acetophenone mixture (1.03 g), 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone and 3,4,5-trimethoxy acetophenone, was added to the reaction vessel. In a separate flask, 6.00 g NaOH was dissolved in 10 mL water with stirring. About 6 mL of this 15 M $NaOH_{(aq)}$ solution was added to the reaction vessel via syringe. The light brown solution turned a reddish yellow-brown upon the addition of the sodium hydroxide solution. The mixture was allowed to stir overnight, and had changed to a dark red-orange color. Some solid had formed on the sides of the flask, but ice was added to the reaction mixture to crash out the expected solid chalcone product. Solid immediately started forming, and this solid was vacuum filtered on a small fritted funnel. The resulting solid was a crystalline-orange solid.

5. Acknowledgements

The author would like to thank Dr. Herman Holt Jr. for his extensive help, patience, and guidance on this research project. The author would also to thank current and former members of the Holt research group for aiding her in learning new laboratory techniques. Finally, much gratitude is expressed towards the University of North Carolina at Asheville Chemistry Department.

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