Proceedings of The National Conference
On Undergraduate Research (NCUR) 2016
University of North Carolina Asheville
Asheville, North Carolina
April 7-9, 2016

Efforts Towards the Synthesis of a Novel Combretastatin A4 Analog with a 5,6,7-Trimethoxy Indole Substituted at the 3-Position

Teresa Rocha
Chemistry
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Herman Holt, Jr.

Abstract

Combretastatin A4 (CA4) is an effective anti-cancer drug that works by inhibiting tubulin formation to decrease cellular growth. Inhibition of tubulin formation also triggers a pathway in the cell that induces a change in cell shape. This deformation of cells in the tumor vasculature system blocks blood flow and selectively kills the tumors. Due to CA4's efficacy against various cancer cell lines, including multidrug resistant cell lines, and due to problems with the drug, such as its low water solubility, further investigations on CA4 analogs are being pursued. Not an uncommon feature for these analogs is the presence of an indole ring. Indoles are highly biologically active molecules and are used in a broad range of pharmaceuticals. Previous research in Dr. Holt's lab group established the need to substitute CA4's trimethoxy benzene ring with a trimethoxy indole, binding at the indole's 2-position. Due to the prominence of indoles in nature being substituted at their 3-position, proposed here is work towards the synthesis of a novel CA4 analog in which the trimethoxy benzene is exchanged for a trimethoxy indole, binding to the *cis*-alkene at its 3-position. This process will be performed utilizing halogenation, coupling and dehydrogenation reactions. Presently, both rings to make the intended analog have been synthesized and work is being pursued towards their coupling and then subsequent reduction to the desired product.

Keywords: Chemotherapy, Cancer, Combretastatin A4, Indole

1. Background

Defined as an umbrella term for many diseases sharing a characteristic cell division abnormality, cancer has reigned as the second highest cause of death for over two decades.[1,2,3] Present treatments have proven insufficient such that cancer rates are predicted to continue increasing in the foreseeable future. A 2015 report by the American Cancer Society predicts an approximate increase of 54% new cases (21.7 million new diagnoses worldwide) and of 59% more reported deaths (13.0 million deaths worldwide) between 2012 and 2030.[3] These statistics support the need for further research into new cancer treatment methods and in improving the current treatment methods which include surgery, radiation, chemotherapy, hormonal therapy as well as combinations of these treatment methods.

Combretastatin A4 (CA4), isolated from the plant *combretum caffrum* in 1988, is a potential chemotherapeutic drug whose more water soluble prodrug is currently undergoing clinical trials.[4] CA4 possesses a similar structure to the known anticancer molecule, colchicine, enabling it to bind at the colchicine binding site on the β-subunit of tubulin thus inducing a similar anticancer effect (see Figure 1). The effect produced by these drugs is a combination of antimitotic (disrupts microtubule formation) and antivasculature (disrupts the tumor's vasculature system).[5] The antimitotic effect causes the cells to halt growth during the M-phase of mitosis (a cellular reproduction cycle) which triggers a self destruct response within the cells.[4] The antivasculature effect is a secondary effect that is induced via inhibition of microtubule formation. This interference in tubulin dynamics activates a Rho protein which triggers the

contemporaneous formation of stress fibers and the rearrangement of actin filaments. These alterations cause the cells to change their shape, becoming more spherical in a process known as membrane blebbing.[6] This blebbing inhibits blood flow through the haphazardly formed tumor's vasculature system which in turn starves the cells of nutrients and results in cell death.[7]

Some defining characteristics of CA4 are that it is less toxic than colchicine, is effective against multi-drug resistant cancer cell lines and induces selective tumor vascular disruption at concentrations well below its max tolerated dose.[8,6,9] CA4 also does not cause bone marrow toxicity or hair loss which are common side effects of other chemotherapeutic drugs.[10]

Some notable shortcomings of CA4 include its water insolubility, which necessitates the use of a more water soluble prodrug (to enable transport through blood), and that the ring of cells attaching the tumor to normal tissue is not affected.[8,11]

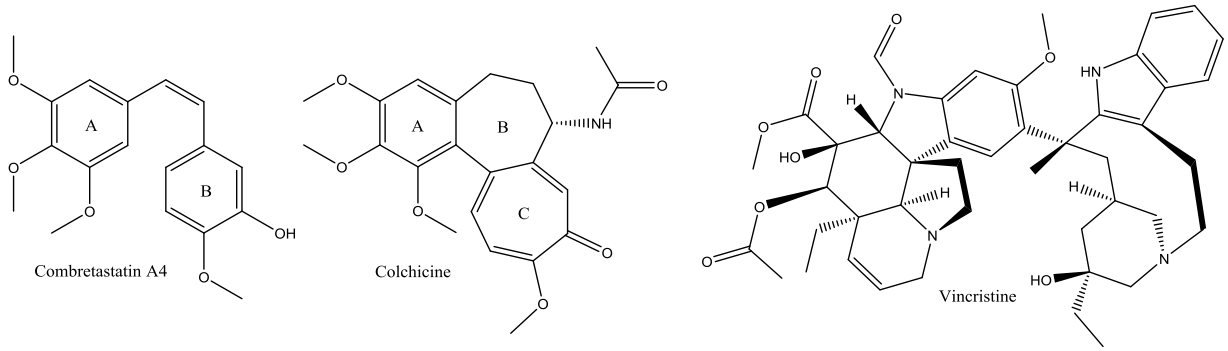


Figure 1. Molecular structure of Combretastatin A4, Colchicine and the vinca alkaloid Vincristine.

Another group of antimitotic drugs, discovered in 1950, are the vinca alkaloids (see Figure 1).[12] The vinca alkaloids were the first antimitotics discovered that contain an indole core and since their discovery, the inclusion of indole cores in chemotherapeutics and pharmaceuticals has become more prevalent (see Figure 2). Some examples of drugs containing indole cores include the clinically used chemotherapeutics vincristine, vinblastine and vinorelobine.[13] This prevalence is likely in part because of the high bioactivity associated with indoles which has promoted their use in pharmaceuticals as anti-depressants, anti-inflammatories, anti-fungicides, anti-tuberculostatics, and antimitotics.[14] Another facet in the prevalence of indoles in chemotherapeutics is their pervasiveness throughout nature, a key example being the essential amino acid L-tryptophan (see Figure 2). Another example is indole-3-carbinol, a molecule associated with the anticancer effect produced by the consumption of cruciferous vegetables (see Figure 2). Unlike the vinca alkaloids, indole-3-carbinol produces its anticancer effect by acting as an antioxidant via up- and down-regulation of various genes. Other benefits found from the inclusion of an indole moiety in chemotherapeutic drugs include an increased toxicity against drug resistant cell lines and a notable decrease in the toxicities associated with standard chemotherapeutics when combined with an indole containing compound.[15]

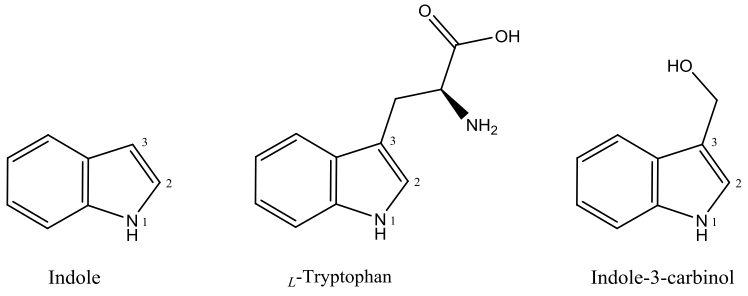


Figure 2. Molecular structure of indole, L-tryptophan and indole-3-carbinol.

Extensive studies have been performed assessing the effects of altering CA4's A-ring, B-ring and its *cis*-alkene bridge. Their findings indicate a more flexible range of modifications for both the B-ring and the bridge (atoms linking the A and B ring together), but a selectively narrow amount of allowed modifications to the A-ring. A-ring

modifications, such as removal of a methoxy, severely diminished or eliminated the molecule's antimitotic effect.[10] Alternatively, work has been done exchanging the phenstatin A-ring (phenstatin is a derivative of CA4) with a trimethoxy indole, illustrating that a bicyclic A-ring can retain antimitotic activity if appropriately substituted with a trimethoxy moiety (see Figure 3).[16,17]

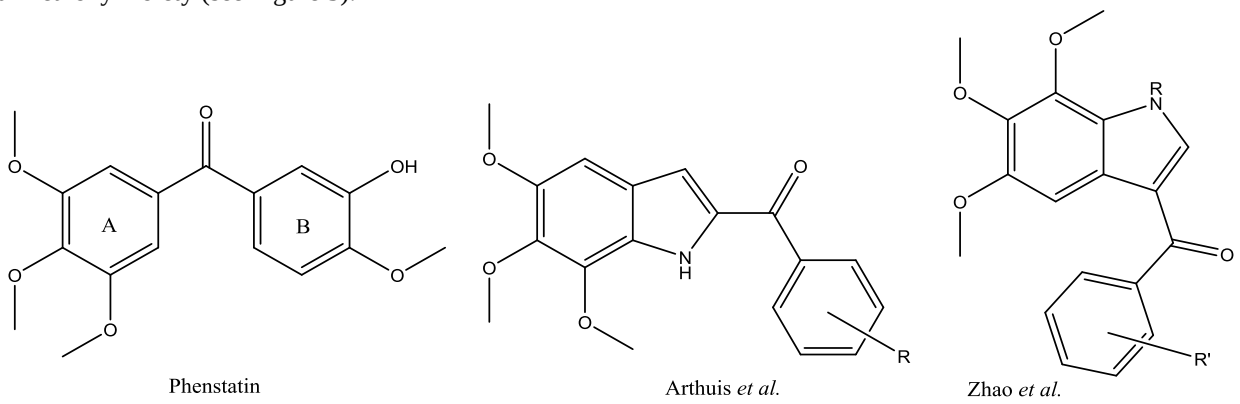


Figure 3. Molecular structure of phenstatin and the general phenstatin indole derivatives.[16,17]

Starting in 2008, prior group member Thomas Graham reported his work towards synthesizing a novel CA4 analog, replacing the A-ring with an N-methyl-5,6,7-trimethoxy indole that was connected to the *cis*-alkene bridge at its 2-position (see Figure 4). In his report he successfully connected the two rings via an alkyne bridge and intended to selectively reduce it to the *cis*-alkene in future work.[18] Building on Graham's work, prior group member Benjamin McDonald in 2012 investigated alternative alkyne reduction pathways and reported the synthesis of a novel N-5,6,7-trimethoxy indole CA4 analog bonded to the *cis*-alkene bridge at its 2-position (see Figure 4).[19] Incorporation of an indole moiety does not negate the problems CA4 is currently experiencing (such as water insolubility) but it may enhance the drug's activity against multidrug resistant cell lines and further reduce the number and severity of side effects experienced due to its use.

Building on the work of past researchers, proposed here is the synthesis of a CA4 analog, replacing the A-ring with a similarly substituted indole linked at its 3-position to the *cis*-alkene (see figure 4). This proposition is based on the prevalence of natural compounds containing indoles substituted at the 3-position and in the hopes of procuring an additive chemotherapeutic effect (see Figure 2). Work towards this goal was previously reported by Rocha in which 5,6,7-trimethoxy indole (the desired A-ring) was successfully synthesized.[20] The final reaction proved inefficient and produced low yields therefore alternative methods were investigated and employed. The updated synthetic scheme is shown below (see Scheme 1).

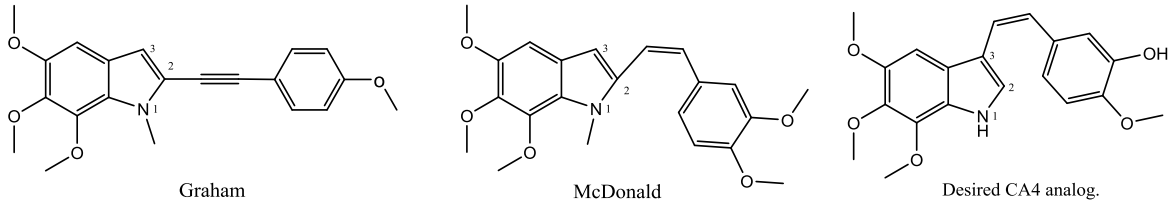
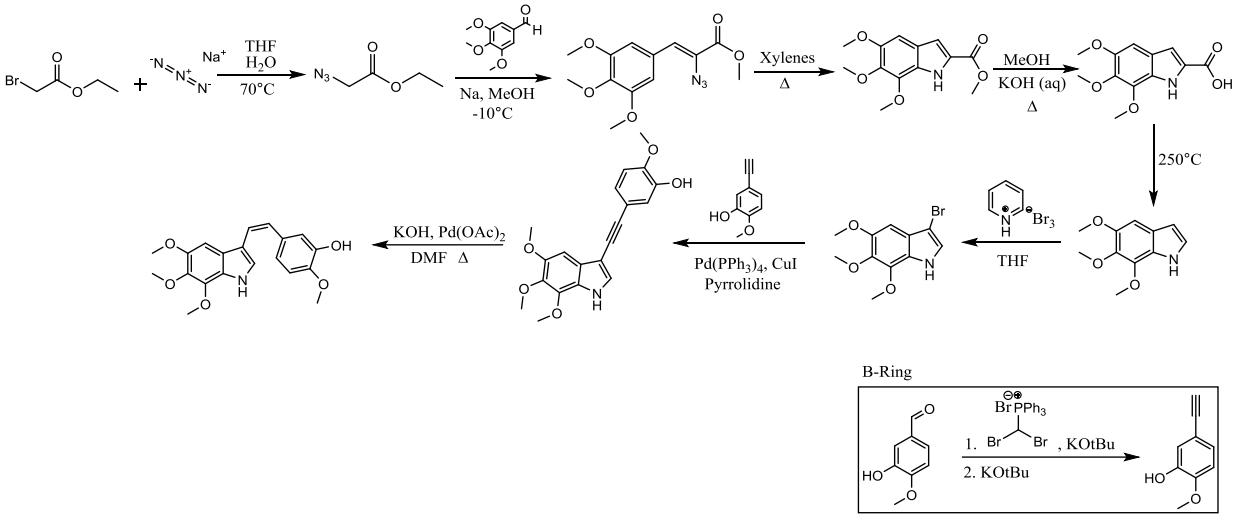


Figure 4. Molecular structure of Graham and McDonald's final products and the proposed target analog.

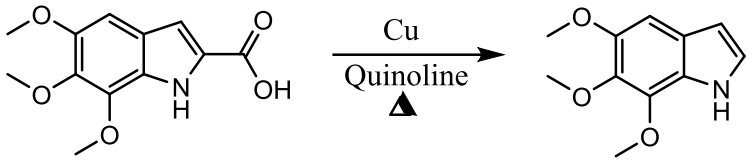
Scheme 1. Proposed synthetic scheme for synthesis of the target CA4 analog.



2. Results and Discussion

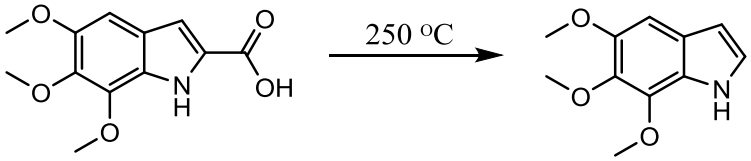
In the work reported by Rocha, 5,6,7-trimethoxyindole-2-carboxylic acid was decarboxylated via refluxing in quinoline with a catalytic amount of copper powder. This resulted in the desired product but only at a maximum of 23% yield (see Scheme 2).[20]

Scheme 2. Decarboxylation of 5,6,7-trimethoxyindole-2-carboxylic acid via copper and quinoline.



Due to the low yields from the copper and quinoline reaction a new method, a solvent-free thermolysis reaction, was proposed (see Scheme 3).[21] This resulted in the desired decarboxylated indole at 36% yield.

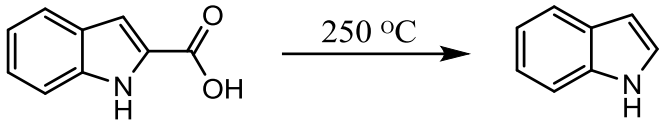
Scheme 3. Decarboxylation of 5,6,7-trimethoxyindole-2-carboxylic acid under solvent-free conditions.



The solvent-free thermolysis reaction provided more efficient reaction conditions as well as a marginally higher yield than the copper and quinoline reaction, therefore an optimization of the solvent-free method was sought. To optimize this reaction a relatively less expensive but similar starting material, 1*H*-indole-2-carboxylic acid, was used and modifications of the reaction conditions were explored (see Scheme 4). Alterations to the surface area of the reaction vessel, the temperature of the reaction and the reaction time were explored as well as the use of a condenser, stirring and of a catalytic amount of base. Only increasing the surface area of the reaction vessel and using a condenser appeared to have any positive effect on the reaction yield. It should be noted that testing the temperature of the sand

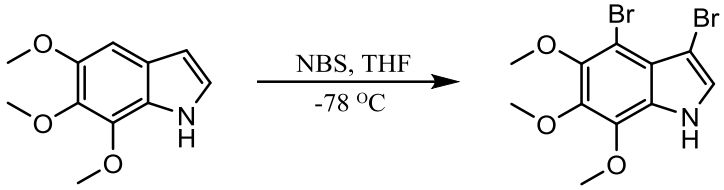
in varying locations revealed that heating was inconsistent throughout the sand bath. The highest yield obtained thus far is 56%.

Scheme 4. Decarboxylation of 1*H*-indole-2-carboxylic acid under solvent-free conditions.



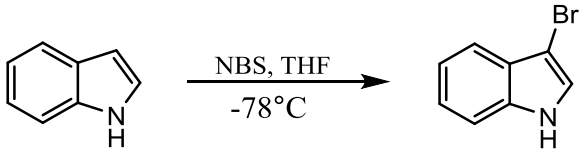
Bromination of the 5,6,7-trimethoxyindole was attempted using NBS as a source of Br_2 (see Scheme 5). The resulting product was analyzed via ^{1}H NMR. When compared to the spectrum for the starting material, protons at the 3 and 4 position of the indole were no longer apparent thus indicating a dibromination to have occurred.

Scheme 5. Bromination of 5,6,7-trimethoxy indole.



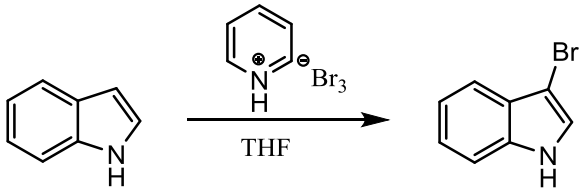
Only a small amount of the trimethoxy indole was available due to the decarboxylation reaction's low yields so it was proposed that 1*H*-indole be used to test how much NBS was necessary in order to make a monobromination product (see Scheme 6). Bromination of the 1*H*-indole was performed using 1.1 eq of NBS, monitoring via thin layer chromatography (TLC) every 10 minutes for an hour. Multiple products were visualized under UV light within the first 10 minutes and these products remained for the hour at which point the reaction was stopped. A second reaction was performed using 0.5 eq of NBS and was monitored via TLC every 10 minutes for an hour. UV visualization of the TLCs revealed a slower rate of product formation but the same number of products formed as for 1.1 eq of NBS by the end of the hour therefore this reaction was also stopped.

Scheme 6. Bromination of 1*H*-indole via NBS.



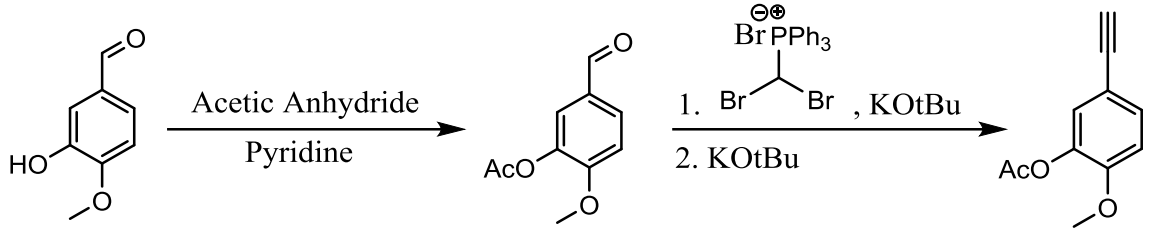
A new brominating reagent was then proposed, pyridinium bromide per bromide, in place of the NBS in hopes of reducing the number of side products formed (see Scheme 7).[22] The previous reaction was repeated except the NBS was replaced with pyridinium bromide per bromide. Although slower than when using NBS, the formation of multiple products was still revealed via TLC by the end of the hour. To check whether these extra products were the result of multiple brominations or of an alternative side reaction, the mixtures were worked up and analyzed via ^{1}H NMR. Comparison of the ^{1}H NMR spectra to that of the starting material revealed loss of the proton peak for the 3-position of the indole, suggesting a monobromination to have occurred.

Scheme 7. Bromination of 1*H*-indole via pyradinium bromide per bromide.



To generate the B-ring (see Scheme 1), an acetylation reaction was used to protect the hydroxy group on 3-hydroxy-4-methoxybenzaldehyde. Acetylation of the 3-hydroxy-4-methoxybenzaldehyde resulted in 47% yield of 5-formyl-2-methoxyphenyl acetate (see Scheme 8). The protected benzaldehyde was then subjected to a Wittig condensation reaction, forming an alkyne from the aldehyde (see Scheme 8). Although the desired formation of the alkyne was confirmed via ^{1}H NMR, so was a partial deprotection of the ring. Further literature research revealed that protecting groups should not be necessary for performing a successful Sonogashira coupling reaction, therefore there was no need to separate the mixture of products.

Scheme 8. Alcohol protection and Wittig condensation of 3-hydroxy-4-methoxybenzaldehyde.



3. Conclusion

Devised here was the synthesis of a CA4 analog replacing CA4's A-ring with a 5,6,7-trimethoxy indole, linked at its 3-position. Presently 5,6,7-trimethoxy indole and 5-ethynyl-2-methoxyphenyl acetate have successfully been synthesized. Progress has been made towards increasing the yields of decarboxylated trimethoxy indole, based on increasing the product yield (+20%) of the 1*H*-indole through modifying the solvent free thermolysis reaction conditions. Efforts have also been undertaken towards the monohalogenation of 5.6,7-trimethoxy indole, by modifying the reaction conditions for bromination of 1*H*-indole (thus testing the reactions with a relatively inexpensive starting material). Proton NMR suggests a monobromination of the 1*H*-indole occurred. From here the modifications that increased yield of the desired test products need to be applied and the remaining proposed reactions outlined in Scheme 1 (Sonogashira coupling of the rings followed by a selective hydrogenation of the combined product) executed in order to synthesize the target novel CA4 analog.

4. Experimental

4.1. General Considerations

Unless stated otherwise, all starting materials were obtained from commercial suppliers and used without further purification. CBr_4 was sublimed prior to use. Anhydrous solvents were either dried over molecular sieves or obtained from an electronic solvent purifier system.
Abbreviations: hexane (Hex), ethyl acetate (EA), dichloromethane (DCM), potassium tertbutoxoide (KOtBU), tetrahydrofuran (THF), acetonitrile (MeCN), N-bromosuccinimide (NBS), deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide (d6-DMSO), sodium bicarbonate ($NaHCO_3$) and magnesium sulfate ($MgSO_4$).

4.2. 5,6,7-Trimethoxy Indole (Solvent-Free Thermolysis Reaction)

A 50.0 mL 2-neck RBF (round bottom flask), stir bar and condenser were baked at 200°C in an oven for one hour and then allowed to cool in open air. The glassware was assembled (but water was not ran through the condenser) and the system closed using septa. A needle was used to vent the system to air. A sand bath was set up using a thermowell and the temperature monitored via a thermometer in the sand. The RBF was placed in the sand bath and heated to 200°C. Next 414 mg of 5,6,7-trimethoxy indole-2-carboxylic acid was added to the flask and agitated with a stir bar. The material was allowed to react for 10 minutes. Some of the resulting material was dissolved with DCM and tested via TLC (70/30 Hex/EA) which indicated that some product was present. The remaining solid was then dissolved with DCM and vacuum filtered through a silica plug into a 1-neck RBF. The DCM was removed via rotary evaporation

followed by subsequent high vacuum removal of any remaining solvent. Proton NMR and IR were used to identify the product material, confirming the formation of 5,6,7-trimethoxy indole (36% yield).

^{1}H NMR (400 MHz, ($CDCl_3$): δ 3.910 (s, 3H), 3.918 (s, 3H), 4.082 (s, 3H), 6.457 (t, 1H), 6.860 (s, 1H), 7.148 (t, 1H), 8.204 (s, 1H).

4.3. Indole (Solvent-Free Thermolysis Reaction)

A sand bath was assembled and a glass vial was partially buried in the sand. The system was closed with a pop cap and then vented with a needle. A stir bar was placed inside the vial. To the vial, 500 mg of 1*H*-indole-2-carboxylic acid was added and then agitated with the stir bar while applying heat. The material was heated for 10 minutes between 200◦C and 220◦C.The vial was removed from heat revealing white crystals which formed along the sides and a hard brown solid along the bottom of the vial. The hard solid was broken up and the white crystals pushed to the bottom of the vial. The vial was buried again in the sand and heated for another 10 minutes between 200◦C and 210◦C. The crystals were transferred to a separate vial, leaving behind a black, hard solid. The vial with the black solid was reacted once more in the sand bath but no white crystals formed. Both the black solid and the previously collected white crystals were dissolved in DCM and rinsed through a silica plug and into a RBF. It should be noted that the black solid did not completely dissolve in the DCM and what remained was disposed of with the silica plug. The DCM was removed from the RBF via rotary evaporation and subsequent high vacuum exposure. Product was confirmed via ^{1}H NMR with 56% yield.

^{1}H NMR (400 MHz, ($(CD_3)_2SO$): δ 6.407 (m, 1H), 6.973 (t, 1H), 7.061 (t, 1H), 7.321 (t, 1H), 7.371 (d, 1H) 7.517 (d, 1H), 11.065 (s, 1H).

4.4. Bromination Of 5,6,7-Trimethoxy Indole

A 50.0 mL 2-neck RBF, air inlet adapter and stir bar were baked in the oven at 200◦C for an hour. The glassware was then assembled and cooled under N_2 gas. Five milliliters of anhydrous THF and 200 mg (0.716 mmol, 1 eq) of 5,6,7-trimethoxy indole were added to the RBF. The solution was stirred and then cooled in an acetone/dry ice bath. To the solution, 133 mg of NBS (0.752 mmol, 1 eq) was added. The reaction was allowed to warm to room temperature after 6 hours and was left to react overnight. The reaction mixture was then extracted with diethyl ether and washed with brine. The organic layer was dried using anhydrous sodium sulfate and then was gravity filtered into a vial. The solvent was removed via rotary evaporation. Analysis of the crude product in $CDCl_3$ was performed via ^{1}H NMR (see Appendix).

4.5. Bromination Of 1*H*-Indole (NBS)

A 50.0 mL 2-neck RBF, air inlet adapter and stir bar were flame dried, assembled and cooled under N_2 gas. Two milliliters of anhydrous THF and 50 mg (0.426 mmol, 1 eq) of 1*H*-indole were added to the RBF. The solution was stirred and then placed into an acetone/dry ice bath. To the solution, 38 mg of NBS (0.213 mmol, 0.5 eq) was added. The reaction was monitored via TLC (70/30 Hex/EA) for an hour and then was stopped and stored in the freezer.

Workup of the reaction was done via extraction with diethyl ether followed by washing with brine. The organic layer was dried using anhydrous sodium sulfate and the mixture was gravity filtered into a flask. The solvent was removed via rotary evaporation. Analysis of the crude product in DMSO via ^{1}H NMR (see Appendix).

4.6. Bromination Of 1*H*-Indole (Pyridinium Bromide Per Bromide)

A 50.0 mL 2-neck RBF, air inlet adapter and stir bar were flame dried, assembled and cooled under N_2 gas. Two milliliters of anhydrous THF and 50 mg (0.426 mmol, 1 eq) of 1*H*-indole were added to the flask. The solution was stirred and then cooled in an acetone/dry ice bath. To the solution, 137 mg of pyridinium bromide per bromide(0.426 mmol, 1 eq) was added. The reaction was monitored via TLC (70/30 Hex/EA) for an hour and then was left to react over the next two days. Diethyl ether and sodium bicarbonate were used to solvate the reaction material and to transfer it to a separatory funnel. The reaction material was then washed with brine and the organic extract dried with

anhydrous sodium sulfate. The resulting mixture was gravity filtered into a flask and the solvent removed via rotary evaporation. Analysis of the crude product in d6-DMSO via ^{1}H NMR (see Appendix).

4.7. 5-Formyl-2-Methoxyphenyl Acetate

An air inlet adapter, 2-neck 100 mL RBF and stir bar were baked at 200◦C for an hour and cooled under N_2 gas. To the RBF, 1.003 g of 3-hydroxy-4-methoxybenzaldehyde was added. Five milliliters of pyridine was then added, stirring to create a solution. Upon complete solvation of 3-hydroxy-4-methoxybenzaldehyde, 1.0 mL of acetic anhydride was added dropwise. The reaction mixture was then allowed to react overnight. The reaction was monitored via TLC (70/30 Hex/EA). Another milliliter of acetic anhydride was added and the solution was left to react overnight. (It should be noted that the acetic anhydride in the initial reagent bottle may have degraded so a new bottle was used.) Another milliliter of acetic anhydride was added and the solution was left to react overnight.

To quench the reaction, 50.0 mL of saturated $NaHCO_3$ was added resulting in a visible reaction. The material was left to stir and react and then more $NaHCO_3$ was added resulting in no visible chemical reaction. The product was extracted with EA (1x 10 mL and 2x 15 mL). The organic layer was then washed with 1M HCl (2x 15mL). The resulting organic layer was washed a final time with 32 mL of brine. The organic extract was dried over anhydrous $MgSO_4$ and then gravity filtered into a 1-neck RBF. Solvent was removed via rotary evaporation. Analysis with ^{1}H NMR confirmed product formation with a crude yield of 47%.

4.8. (Dibromomethyl)Triphenylphosphonium Bromide

A 100.0 mL 2-neck RBF, an air inlet adapter, and a stir bar were baked at 200◦C for an hour in the oven. The CBr_4 was sublimed using a cold finger. The glassware was removed from the oven, assembled in the hood and cooled under N_2 gas. The system was closed with a septum. To the RBF, 15.0 mL of dry DCM and 1.582 g of PPh_3 were added. The mixture was then agitated via the stir bar until complete solvation of the PPh_3 occurred. Four portions of CBr_4 were measured (totaling 1.0 g) and a portion was added every 2-3 minutes. The solution turned from clear to yellow to red upon subsequent additions of the CBr_4. The solution was allowed to react for approximately 20 minutes and then 4.0 mL of deionized H_2O was added dropwise, changing the solution back to a clear yellow color. The mixture was then separated using a separatory funnel and the organic layer dried over anhydrous $MgSO_4$. The solution was then gravity filtered and cooled in an ice bath. MeCN was added dropwise to the cooled solution while swirling. A white precipitate crashed out. The white powder was collected using a fine glass fritted funnel and subsequently dried under vacuum. Analysis via ^{1}H NMR confirmed product formation (17% yield).

4.9. 5-Ethynyl-2-Methoxyphenyl Acetate

A 50.0 mL 2-neck RBF, air inlet adapter, and stir bar were baked in the oven at 200◦C for an hour. The glassware was assembled and then cooled under N_2 gas. To the flask, 35 mg (0.008 mmol, 2 eq) of (dibromomethyl)triphenylphosphonium bromide was added along with 5 mL of anhydrous THF. The mixture was stirred. To the stirring mixture in the flask, 9 mg of KOtBU (0.008 mmol, 2 eq) was added and allowed to mix for 5 minutes. Next, 8 mg of 5-formyl-2-methoxyphenyl acetate was added and the reaction monitored via TLC. After 30 minutes starting material still remained so 11 mg of KOtBU was added. After another 30 minutes, 10 more mg of KOtBU was added to the reacting mixture. The material was then allowed to react overnight. The following morning 28 mg of KOtBU was added to the reaction mixture and after 10 minutes the system was quenched with brine. The reaction mixture was then extracted (x2) with diethyl ether and dried with sodium bicarbonate. The resulting mixture was gravity filtered into a flask and the solvent removed via rotary evaporation. Analysis of the crude product via ^{1}H NMR indicated that successful alkyne formation occurred along with partial deprotection of the alcohol functional group.

5. Acknowledgements

The author extends thanks to Dr. Herman Holt Jr. and the Holt Research group for their guidance and support. Thanks are also extended to the National Science Foundation and University of North Carolina Asheville's Undergraduate

Research Program for their financial support as well as to the University of North Carolina Asheville's Chemistry department for making this research possible.

6. References

1 National Cancer Institute. www.cancer.gov

2 Springhouse Corporation. Neoplasms. In *Professional Guide to Diseases*, 5th ed.; PA: Cahill, Mathew, 1995; pp 38-139.

3 Murphy, S.L.; Xu, J.; Kochanek, K.D. *National Vital Statistics Report* **2013**, *61*, 1-118.

4 Liou, J.-P.; Chang, Y.-L.; Kuo, F.-M.; Chang, C.-W.; Tseng, H.-Y.; Wang, C.-C.; Yang, Y.-N.; Chang, J.-Y.; Lee, S.-J.; Hsieh, H.-P. *J. Med Chem.* **2004**, *47*, 4247-4257.

5 Dark, G.G.; Hill, S.A.; Prise, V.E.; Tozer, G.M.; Pettit, G.R.; Chaplin, D.J. *Cancer Res.* **1997**, *57*, 1829-1834.

6 Kanthou, C.; Tozer, G. *Blood* **2002**, *99*, 2060-2069.

7 MacDanough, M.; Strecker, T.; Hamel, E.; Hall, J.; Chaplin, D.; Trawick, M.; Pinney, K. *Bioorgan. Med. Chem.* **2013**, *21*, 6831-6843.

8 Tron, G.C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A.A. *J. Med. Chem.* **2006**, *49*, 3033-3044.

9 Flynn, B.L.; Gill, S.G.; Grobelny, D.W.; Chaplin, J.H.; Paul, D.; Leske, A.F.; Lavranos, T.C.; Chalmers, D.K.; Charman, S.A.; Kostewics, E.; Shackleford, D.M.; Morizzi, J.; Hamel, E.; Jung, M.K.; Kremmidiotis, G. *J. Med. Chem.* **2011**, *54*, 6014-6027.

10 Aprile, S.; Del Grosso, E.; Grosa, G. *Xenobiotica* **2009**, *39*, 148-161.

11 Rustin, G.J.; Shreeves, G.; Nathan, P.D.; Gaya, A.; Ganesan, T.S.; Wang, d.; Boxall, J.; Poupard, L.; Chaplin, D.J.; Stratford, M.R.L.; Balkissoon, J.; Zweifel, M. *Brit. J. Cancer* **2010**, *102*, 1355-1360.

12 Sidhu, J.; Singla, R.; Jaitak, M.; Jaitak, V. *Anti-Cancer Agents in Medicinal Chemistry* **2016**, *16*, 160-173.

13 Kumar, N.; Kumar, D. *Chemistry and Biology Interface* **2013**, 276-303.

14 Panda, S.; Tripathy, J.K. *J. Chem. Pharm. Res.* **2010**, *2*, 722-732.

15 Ahmad, A.; Sakr, W.; Rahman, K.M. *Current Drug Targets* **2010**, *11*, 652-666.

16 Arthuis, M.; Pontikis, r.; Chabot, G.G.; Quentin, L.; Scherman, D.; Florent, J.-C. *Eur. J. Med. Chem.* **2011**, *46*, 95-100.

17 Zhao, D.-G.; Chen, J.; Du, Y.-R.; Ma, Y.-Y.; Chen, Y.-X.; Gao, K.; Hu, B.-R. *J. Med. Chem.* **2013**, *56*, 1467-1477.

18 Graham, T. (2008). Design and Progress Toward the Synthesis of a Novel Indole-based Combretastatin A4 Analogue. B.S. Thesis. University of North Carolina at Asheville: U.S.

19 McDonald, B. (2012). Novel Route to the *cis*-Combretastatin Indole Analogs via Selective Partial Reduction of Arylethynyl Indoles. B.S. Thesis. University of North Carolina at Asheville: U.S.

20 Rocha, T. *Proceedings of the National Conference on Undergraduate Research 2015*.

21 Conversation with Thomas Graham.

22 Conversation with Dr. Herman Holt Jr.