University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2020

Synthesis of Triazoles as Combretastatin A-4 Analogs

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Abstract

Combretastatin A-4 (CA-4) has been shown to have anticancer properties by preventing mitosis. However, the active *cis*-conformation of these molecules are thermodynamically unstable, making it difficult to maintain their activity. Additionally, water solubility and non-specific binding create a need for alternatives to CA-4 drugs. Indole analogs of CA-4 that contain triazoles can be synthesized as a way to create *cis*-restricted molecules with similar anticancer properties. The triazole takes the place of the cis-alkene and restricts the analog to the *cis*-conformation. In this experiment, the preparation of vinyl azides will be done via condensation reactions of trimethoxybenzaldehyde and azidoesters, followed by treating them with xylenes to form indoles. The molecules will then be homologenized to create ethynyl indoles and reacted with benzyl azide to form triazoles. The biological activity of these synthesized molecules will be measured and compared to others in the literature to determine their effectiveness. To date, ethylazidoacetate was synthesized from ethylbromoacetate. Several reactions to form vinyl azide were attempted with varying unsuccessful results.

1. Introduction

Anticancer drugs as an alternative treatment to radiation have been increasingly prevalent as new types of drugs are developed. Although cancer has been one of the world's major health problems for a long time, it continues to present difficulties in effective treatments. Tumor cells are difficult to eradicate, and treatments must be potent enough to kill cancerous tumor cells with minimal damage to healthy cells. Some treatments have severe side effects and are not always effective at treating cancer cells, so new treatments must be pursued.

Antimitotic drugs specifically target the microtubules in cells, which prevents mitosis from occurring, leading to cell death. The prevention of mitosis is done by the binding of the drug to tubulin at the colchicine receptor. Combretastatin A-4 (CA-4, Figure 1) can be isolated from the tree *Combretum caffrum* and has displayed a potency against multiple cancer cells. In addition, it has also been shown to restrict blood flow to tumor cells.[1] However, there is little bioavailability of this molecule, and efficient synthesis schemes in laboratories are necessary to produce this drug on a larger scale. Modifications to make the drug more potent are also needed to help combat drug-resistant lines of cancer cells.

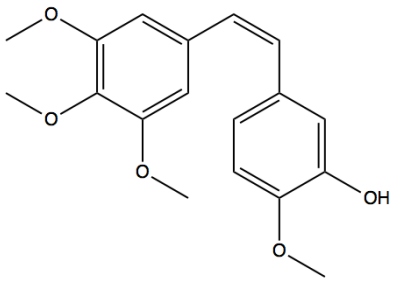


Figure 1: Combretastatin A-4

One challenge with CA-4 is that it is only potent in the *cis* configuration, although the *trans* configuration is much more stable. The synthesis of different heterocyclic analogues has been shown to be one effective way to combat the issue of instability. In addition, issues with low water solubility have begun to be addressed by modifying combretastatin analogs.[2]

Both triazole and indole rings are prevalent in several areas of medicine. Antimicrobials, analgesic agents, antivirals, anti-Parkinson's, and antidiabetics are a few areas of medicinal agents in which triazole-containing molecules have been utilized. Triazoles are heterocyclic compounds that are soluble in both water and alcohol. Triazole medicinal agents also display high levels of potency.[3] Replacing the ethene bridge in CA-4 with a triazole forces the molecule to be in a *cis* conformation. Triazoles have shown bioactivity as anticancer drugs (Figure 2). Because of the *cis* like conformation, these molecules mimic CA-4 and target the colchicine binding site. The goal of this research project is synthesizing triazole indole analogs of Combretastatin A-4.

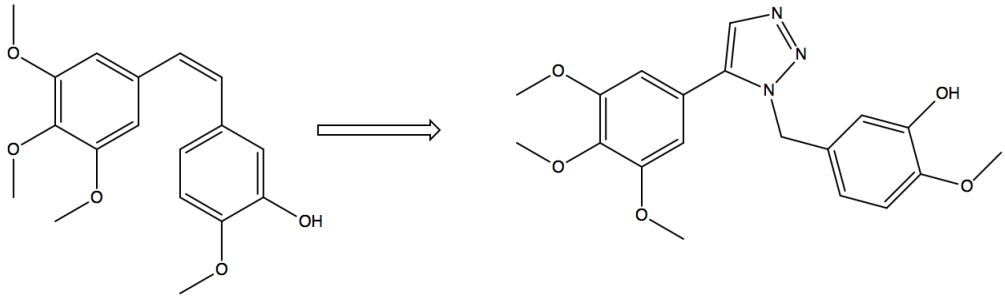


Figure 2: Example of Triazole Analog of CA-4

Benjamin Shields and Dr. Herman Holt of the University of North Carolina at Asheville successfully synthesized three analogs of CA-4. Triazole indole analogs were synthesized in an attempt to mimic the *cis* double bond of CA-4. The analogs were synthesized in moderate yields, but no testing was done to determine anti-tubulin activity.

In Shields' research, structure activity relationship (SAR) studies were used to determine the correlation between molecule structure and biological activity. Proposed analogs were compared to SAR studies found in the literature to hypothesize the binding strength of the molecules into the active site.

Shields synthesized a few different derivatives of CA-4, including analogs with ketone linkages, triazole linkages, indole analogs with the cis olefin bridge, and triazole indole analogs. Vinyl azides were prepared and converted to indoles. The molecules were then homologenized to create ethynyl indoles and reacted with benzyl azide to form triazoles.[4]

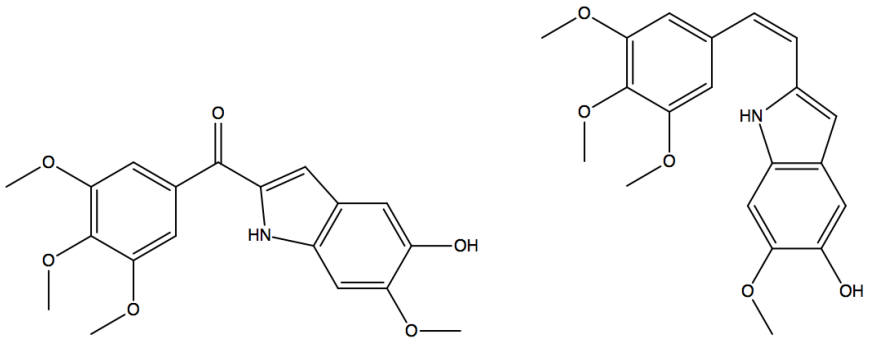


Figure 3: Analogs proposed by Shields: Ketone indole linkage (left) and cis olefin indole (right)

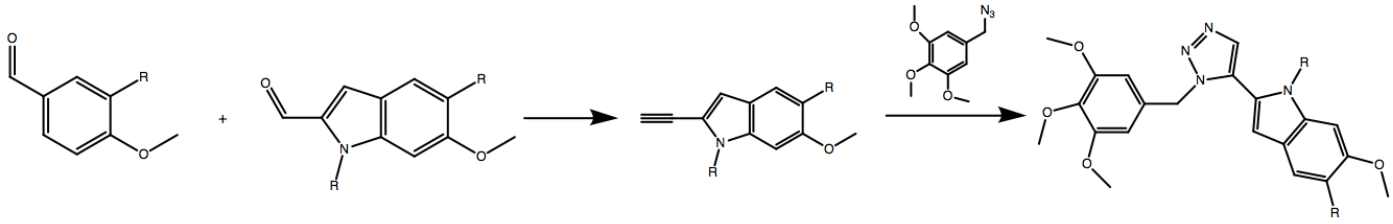


Figure 4: General synthesis of triazole indole analogs

Another research group examined combretastatin-like chalcones (shown in figure 5) to determine if these analogs had similar potency towards cancer cells as CA-4 analogs. The chalcone analogs are simple to synthesize, which shows promise as an alternate and simpler way to synthesize combretastatin-like analogs.[5]

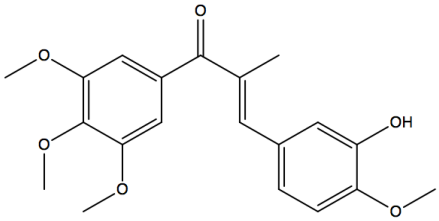


Figure 5: Combretastatin-like chalcone analog

A research group consisting of Kristin Odlo, Jérémie Fournier-Dit-Chabert, and Sylvie Ducki also synthesized CA-4 analogs with a triazole linkage. Several molecules were synthesized and tested for cytotoxicity and binding energies to the colchicine binding site. Triazoles with shorter bridge lengths were found to be more cytotoxic.[6] As shown in figure 6, increasing the number of carbons lengthens the bridge length between the cyclic rings in the molecule.

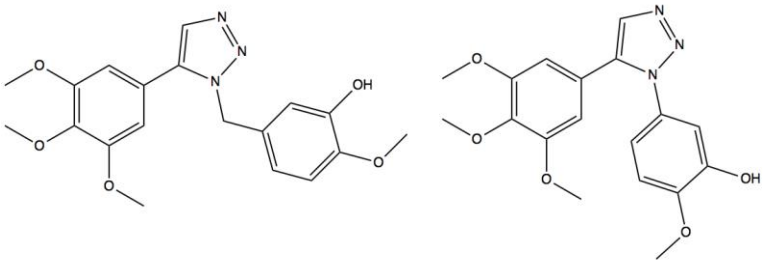


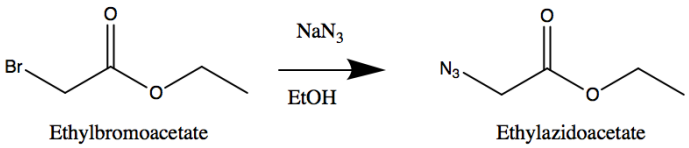
Figure 6: Triazole analogs with different bridge lengths

To date, CA-4 analogs are the most potent of the combretastatin derivatives, and major issues lie in finding ways to improve water solubility and improving stability of the *cis* conformation.[7] Modifying substituents on CA-4 as well as examining the potency of similar molecules are important to the future of this field of research.

2. Results and Discussion

2.1 Synthesis of ethylazidoacetate

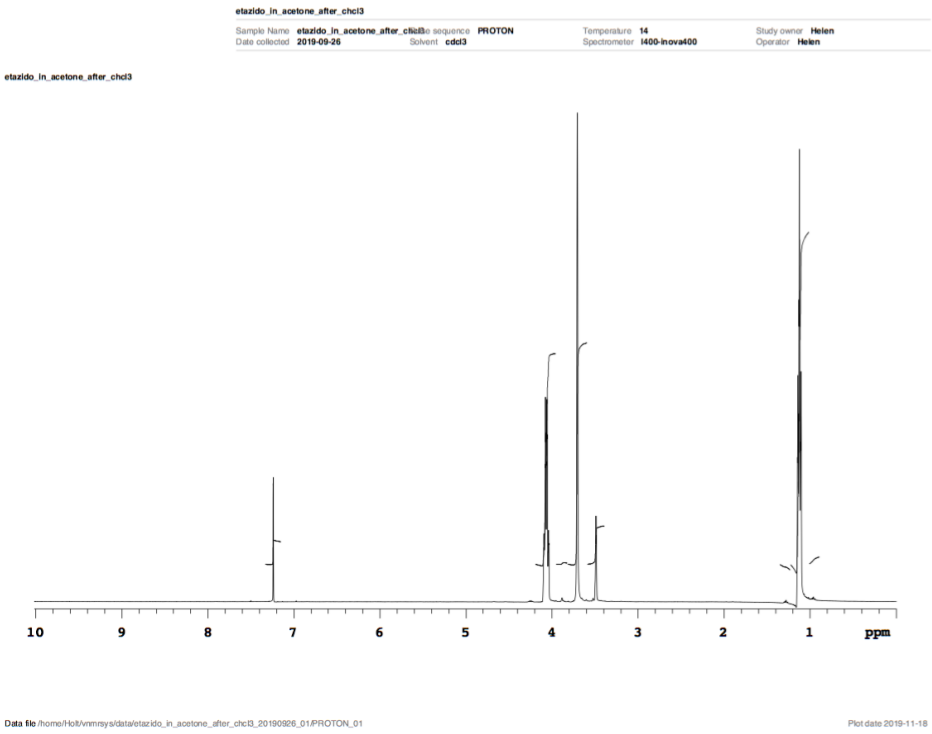
In order to add an azido group, ethyl bromoacetate (50 mL, 0.452 mol) and a slurry of sodium azide (42.5 g, 0.654 mol) with 40.0 mL water were combined in ethanol (86.0 mL) in a 500 mL round bottom flask with a magnetic stir bar and a reflux condenser. The contents were stirred for 20 minutes at room temperature and then refluxed gently for 2 hours. The solvent was removed on the rotavapor (25 °C water bath). To a separatory funnel, the residue of the round bottom flask, 100 mL Et_2O and 100 mL H_2O were added. The aqueous layer was extracted with Et_2O (3 x 30 mL). The organic layers were combined and dried with $MgSO_4$ and filtered. The reaction of ethyl bromoacetate and sodium azide was extracted in a separatory funnel after the solvent was removed three times due to an excess amount of water remaining in the organic layer. The dried organic layer was left to evaporate and then rotovapped to remove solvent.[7] Following an inability to get all solvent out of the reaction, the remaining product was extracted twice more using Et_2O (3 x 30 mL). After evaporation, the product was a yellow oil. An ^{1}H-NMR was taken and was found to be inconclusive. The repetition and unsuccessfulness of this step may have led to an impurity in the product, leading to an inconclusive ^{1}H-NMR.



Ethylbromoacetate Ethylazidoacetate

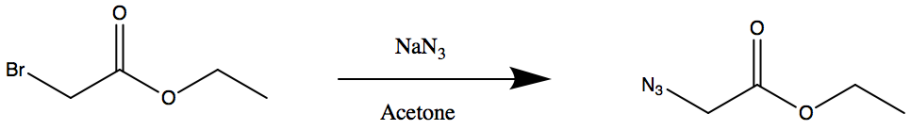
Scheme 1: Reaction with ethanol to form ethyl azidoacetate

A second reaction was carried out in a 250 mL 3-neck round bottom flask fitted with a stir bar and condenser under nitrogen. Sodium azide (1.30 g, 0.020 mol) was added to the flask and rinsed with a few milliliters of water. More water (15.0 mL) was added to the reaction which was stirred until the solution turned a tan color. Ethyl bromoacetate (1.10 mL, 0.00995 mol) was added to the solution using a syringe, followed by 50.0 mL of acetone and 50.0 mL of water. The solution was stirred at room temperature overnight and turned a pale yellow. To the solution, 20.0 mL water was added followed by a liquid-liquid extraction using ethyl acetate (3x15.0 mL). The organic layers were combined and washed with 25.0 mL of brine and dried with $MgSO_4$. The solution was filtered and rotovapped to produce a pale-yellow liquid. To further purify the product after an ^{1}H-NMR was taken, 30.0 mL chloroform was added to the flask and the solution was rotovapped. A second ^{1}H-NMR was taken and showed the product to be pure.



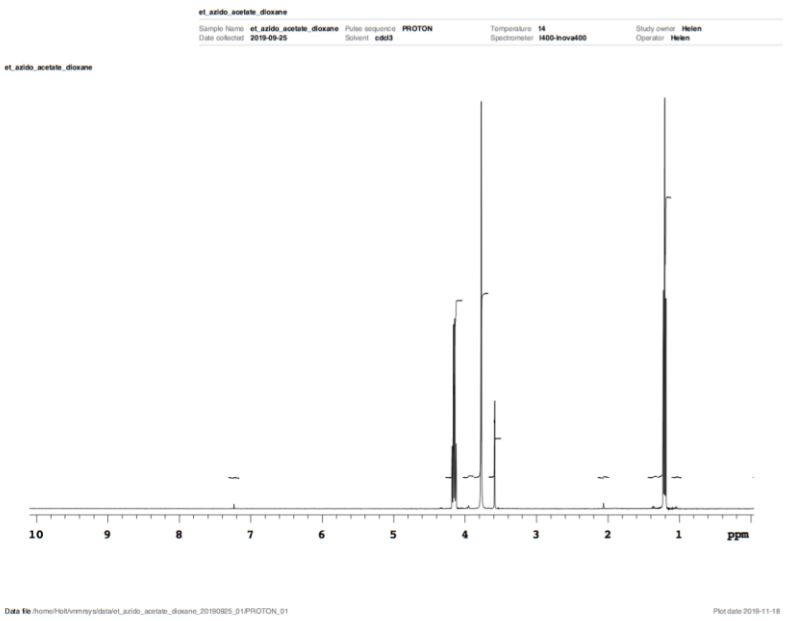
2.2 Method A: Acetone

The second attempt was run using acetone as the solvent in order to more easily evaporate the solvent out of the reaction. An ^{1}H-NMR showed the product to have slight impurities. The addition of chloroform followed by concentrating under reduced pressure cleaned up the spectrum which clearly showed a triplet at 1.1 ppm, a singlet at 3.7 ppm, and a multiplet at 4.1 ppm, confirming the presence of the desired product by ^{1}H-NMR.



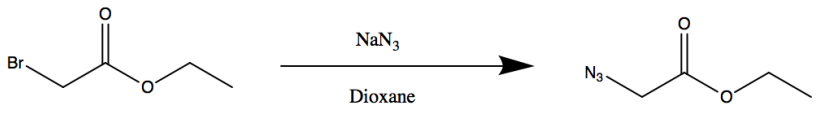
Scheme 2: Reaction with acetone to form ethyl azidoacetate

A similar reaction was completed in which 20.0 g sodium azide (0.308 mol) and water (22.0 mL) were added to a 250 mL two-neck round bottom flask. This solution was stirred for 10 minutes at room temperature before the addition of 50.0 mL dioxane (0.584 mol) at 50 °C. Ethyl bromoacetate (16 mL, 0.145) was added to the stirring solution which was then heated to 80 °C and refluxed for 72 hours. After the solution turned orange, the flask was taken off reflux and allowed to cool. The solution was filtered, and a liquid-liquid extraction was performed with diethyl ether (3 x 20.0 mL). The organic layer was washed with brine and dried with $CaCl_2$. The product was a yellow liquid. The presence of the desired product was confirmed by ^{1}H-NMR.



2.3 Method B: Dioxane

The reaction of ethyl bromoacetate and sodium azide in dioxane was refluxed at 80 °C. An ^{1}H-NMR was taken yielding a triplet at 1.2 ppm, a singlet at 3.8 ppm, and a multiplet at 4.2 ppm, confirming the presence of the desired product.

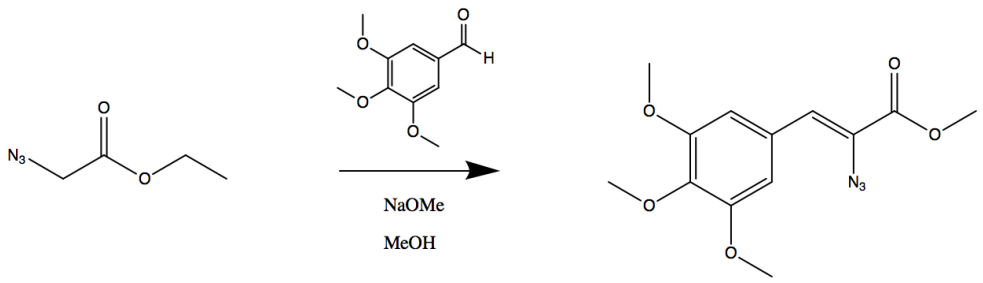


Scheme 3: Reaction with dioxane to form ethyl azidoacetate

2.3.1 synthesis of vinyl azide

A three-neck round bottom flask with a condenser and addition funnel over a dewer of dry ice and methanol was added 3.8 mL dry MeOH and 0.30 g sodium metal (0.013 mol) under nitrogen. Into a single neck round bottom flask, 0.33 g of 3,4,5-trimethoxybenzaldehyde (0.00172 mol), 0.5 mL ethyl azidoacetate (0.00434 mol), and 1.5 mL dry MeOH were added under nitrogen. The contents of the 1 neck flask were swirled and syringed into the addition funnel and added dropwise. Once the addition was complete, the 3-neck flask was covered in foil, warmed to room temperature, and left overnight. The contents of the flask were poured into a 250 mL beaker containing crushed ice and saturated NH_4Cl. The solution was stirred overnight. A liquid-liquid extraction was performed with diethyl ether, the organic layers were washed with brine, and dried with sodium sulfate. To the aqueous layer was added HCl to reduce the solution to a pH of 1, and the extraction process was repeated to yield an orange oil. An ^{1}H-NMR indicated the presence of unreacted benzaldehyde with a peak around 10 ppm.

The reaction to form a vinyl azide was performed twice more in an attempt to isolate pure vinyl azide. In each reaction, the presence of unreacted benzaldehyde was indicated by an ^{1}H-NMR. The acidification and refiltration were completed in an attempt to isolate the product.



Scheme 4: Reaction to form vinyl azide

3. Conclusion

The ultimate goal of this project was to prepare triazole indole analogs of combretastatin A-4. Ethylazidoacetate was successfully synthesized via S_N2. Despite several attempts at synthesizing vinyl azide, a pure product was unable to be isolated. Future efforts would be focused on optimizing reaction conditions to form vinyl azide and completing the rest of the scheme to form the intended product.

4. References

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