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Synthesis of Pyrazoline Derivatives from Chalcones

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Abstract

Combretastatin A-4 (CA-4) has long been used as an anti-mitotic agent; however, CA-4 is not a favorable drug for use in anti-cancer treatments, due to its high solubility in fats and its low solubility in aqueous media. Chalcones, which are derivatives of CA-4, have been found to have anti-bacterial and anti-cancer responses and can be further modified into various heterocyclic compounds. In this study, derivatives of the nitrogenous heterocyclic compound, pyrazoline, are the focus. Previous research has shown pyrazoline derivatives to be effective against various cancer cell lines. These molecules can be synthesized from the cyclization of chalcones by using an aryl aldehyde, along with hydrazine hydrate. Specifically, this project is concerned with the aza-Michael addition of hydrazine to synthesize pyrazoline derivatives using a "solvent free" method using the ionic liquid, [DBU][Ac] (1,8-diazabicyclo[5.4.0]-undec7-en-8-ium acetate). The use of [DBU][Ac] has been proven to be a good catalyst for aza-Michael addition reactions and can provide high yields. Three aza-Michael addition reactions have been attempted. However, all three syntheses resulted in a hydrazone byproduct, confirmed by comparison to the ^{1}HNMR of a previously synthesized hydrazone. In addition, chalcone, [DBU][Ac], and benzylamine were reacted in order to gain more insight on the reactivity of the chalcone.

1. Introduction

Cancer is one of the most important health issues in developed nations, with an estimated 18.1 million new cases in 2018 along with 9.6 million deaths.[1] Despite its commonality, cancer is still incredibly challenging to treat, hence the need for continuing research regarding treatments. Much work has been done in the field of organic synthesis of new compounds that have cytotoxic properties. One such compound is combretastatin A-4 (CA-4), whose structure can be seen in figure 1. CA-4 selectively disrupts the abnormal tumor vasculature by binding to the protein tubulin, which is related to cellular functions such as mitosis.[2,3] Molecules such as CA-4 are classified as antimitotic agents, meaning any drug that blocks the growth of cells by interrupting the division of cells. Antimitotic drugs inhibit the polymerization of microtubules by activating the spindle assembly checkpoint (SAC), therefore blocking transition from metaphase to anaphase.[4] Despite CA-4's effective cytotoxic properties, it is not favorable for use as an anticancer drug, due to its high solubility in fats and its low solubility in aqueous media.[2] Development of new compounds that interrupt mitosis in tumor cells without interfering with microtubule dynamics in healthy cells is the primary goal in new antimitotic drug research and development.[4] The study and discovery of new antimitotic compounds is important due to the fact that many current antimitotic drugs are not very effective against multidrug resistant (MDR) cell lines, have many side effects, and have difficult dosing schedules.[5]

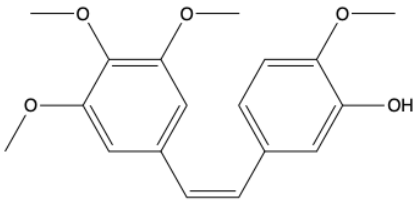
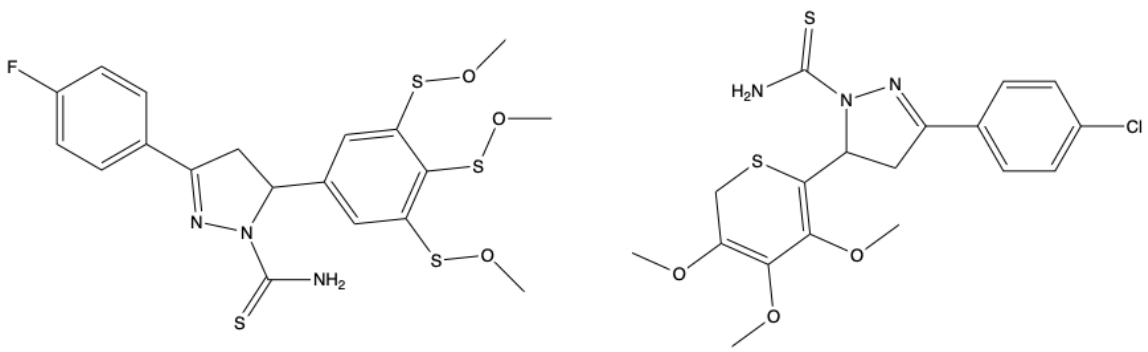


Figure 1: Structure of Combretastatin A4 (CA-4).

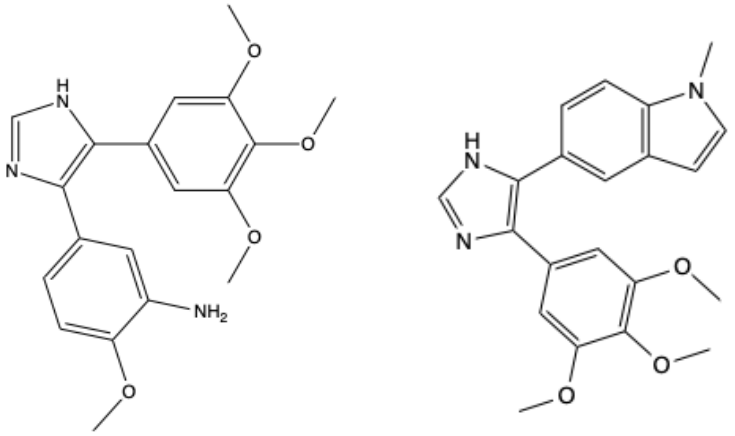
Chalcones, which are similar in structure to CA-4, have been studied by the Holt lab because of their anticancer responses. Similarly to combretastatin A-4, chalcones interfere with tubulin.[3] Chalcones can be further modified into heterocyclic compounds. One of these heterocyclic compounds is pyrazoline. Pyrazoline has been established as a promising molecule against cancer cell lines. Synthesized compounds found below in figure 2 showed good activity against HepG-2 cancer cells. Their IC_{50} values, meaning the concentration of a drug required for 50% inhibition, were 6.78 μM (A) and 16.02 μM (B). Both values were comparable to the IC_{50} value for cisplatin (7.57 μM), a known anticancer drug currently on market. These compounds were also tested on normal NIH/3T3 cells, with IC_{50} values ranging from 23.52 μM to 100 μM. These results can confirm that the tested pyrazoline derivatives are less cytotoxic to normal, healthy cells than cisplatin.[6] SAR studies have shown that the heterocyclic nitrogen functional group is imperative for antitumor activity. Wang's research on pyrazoline derivatives of chalcones has shown that analogs of pyrazoline that contain ethoxy groups on their phenyl moieties have the most promising activity against Hela cells.[6]



[A] **[B]**

Figure 2: Synthesized pyrazoline derivatives which showed good activity against HepG-2 cancer cells. (IC_{50}: 6.78 μM [A], 16.02 μM [B])

Additionally, heterocycles have also been studied in regard to combretastatin A-4. In another study, various molecules with heterocycles replacing the *cis* alkene in CA-4 were synthesized. The best replacement for the *cis* alkene was found to be 4,5- disubstituted imidazole. Additionally, 3-amino-4-methoxyphenyl (3A) and N-methyl-indol-5-yl were discovered to be the best replacements for the 3-hydroxy-4-methoxyphenyl in CA-4.[5] Two of these synthesized molecules, shown in figure 3, were found to have potent cytotoxic and antitubulin activity. Cytotoxicities of the heterocycle-based CA-4 analogs were evaluated against NCI-H460 and HCT-15 cancer cell lines. IC_{50} values against NCI-H460 cells were 8.5 nM (A) and 51 nM (B). IC_{50} values against HCT-15 cells were 8.1 nM (A) and 27 nM (B). The substitution pattern of the imidazole ring was found to play an important part in the interference of tubulin.[5] It has been determined that the most effective substitution pattern for potency is 4(5),5(4)-disubstituted imidazole. Furthermore, the addition of an N-methyl group on the imidazole ring (figure 4) improves the pharmacokinetics, although it does lead to a decrease in cytotoxicity and antitubulin activity.[5]



[A] **[B]**

Figure 3: Previously synthesized imidazole-based CA-4 analogs shown to have cytotoxic properties.

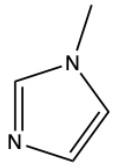


Figure 4: General structure of an N-methyl imidazole.

More specifically, this research project will focus on synthesizing pyrazoline derivatives from chalcones using a solvent free method. To do this, [DBU][Ac] will be used as an ionic liquid in place of a traditional organic solvent. An ionic liquid is a salt which contains poorly coordinated ions which in turns results in the solvents remaining as liquids below 100 °C. The main benefit of using this procedure is the shortened reaction time.[7] Once these pyrazoline derivatives have been made, MTT bioassays can be performed in order to measure the cell viability, likely using Hela cancer cells. Doing so will yield IC_{50} values, and from these values further modifications to the pyrazolines can be determined.

2. Results & Discussion

The synthesis of (2E)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (figure 5) was attempted three times, although the results were unsuccessful. The first attempt involved acetophenone, benzaldehyde, 6 M NaOH, and ethanol. The second attempt involved acetophenone, benzaldehyde, 50% aqueous KOH, and ethanol. The third used the method of first attempt, although the benzaldehyde was added to the mixture an hour after the rest of the reagents were added. ^{1}H-NMR data was acquired for all products from these syntheses. The signals present for each suggest that the chalcone was not successfully synthesized, and determined that the hydroxy proton on the benzaldehyde was deprotonated instead of the protons on the acetophenone to allow the reaction to proceed appropriately. To remedy this issue, the 4-hydroxybenzaldehyde was protected by using 3,4-dihydropyran (DHP), pyridinium p-toluenesulfonate (PPTS) as a catalyst, and dichloromethane (DCM) as the solvent. Its structure can be seen below in figure 6. ^{1}H-NMR data confirmed the synthesis of the protected benzaldehyde. The chalcone was successfully synthesized using the protected benzaldehyde after two attempts. The first unsuccessful attempt involved the addition of the protected benzaldehyde an hour after the rest of the reagents were added, and the use of ethanol as a solvent. The resulting product from this reaction was determined to be the starting acetophenone. The second, successful, attempt similarly involved the late addition of protected benzaldehyde after an hour but used methanol as

the solvent. A hypothesis as to why methanol was more successful than ethanol as the solvent is due to methanol's higher dielectric constant. Since the workup to this reaction involved the addition of ice to cause the product to crash out of solution, a higher dielectric constant for the solvent would mean that the product could crash out more easily. The chalcone was characterized by ^{1}H-NMR, which confirmed its synthesis.

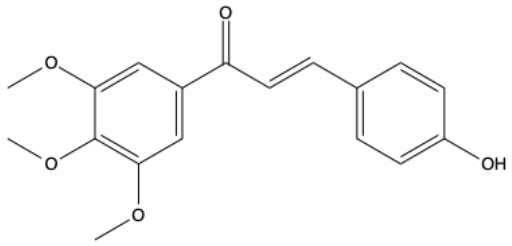


Figure 5: Structure of (2E)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one

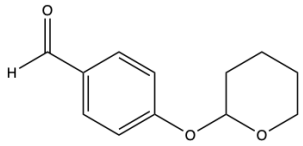


Figure 6: Structure of 4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde.

Three different pyrazoline derivatives were attempted, although unsuccessfully synthesized. Their structures can be seen in figures 7, 9 and 10. The first attempt involved stock (2E)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, hydrazine monohydrate, unsubstituted benzaldehyde, and [DBU][Ac]. The second involved stock (2E)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, hydrazine monohydrate, p-anisaldehyde, and [DBU][Ac]. The third involved the chalcone synthesized from the DHP protected benzaldehyde, hydrazine monohydrate, unsubstituted benzaldehyde, and [DBU][Ac]. All reactions were stirred overnight at room temperature. ^{1}H-NMR data was also acquired for all pyrazoline derivatives, with all three having a sharp signal at 8.6 ppm, which suggested that hydrazone byproducts were made. Hydrazone (figure 8) was purposefully synthesized in order to confirm that hydrazone was in fact the product of these reactions. Further synthesis of pyrazoline derivatives can be confirmed by comparison of the ^{1}H-NMR data for the hydrazone and the pyrazoline derivative. The synthesized chalcone, [DBU][Ac], and hydrazine were reacted in order to gather results of the reaction without the benzaldehyde, eliminating the possibility of a hydrazine byproduct. ^{1}HNMR data was acquired for this reaction and suggested that the product was benzaldehyde, indicated by a peak at 9.9 ppm. Additionally, the synthesized chalcone, [DBU][Ac], and benzylamine were all reacted in order to test the reactivity of the chalcone. TLC of this reaction showed two spots, and purification of the resulting product was performed via column chromatography. ^{1}HNMR data suggested that the resulting compound from the column was the starting benzaldehyde. These results of synthesizing the initial benzaldehyde were unexpected, as it was not present as a starting material in either reaction. It is hypothesized that either the [DBU][Ac] or amine is reverting the chalcone back to the starting benzaldehyde.

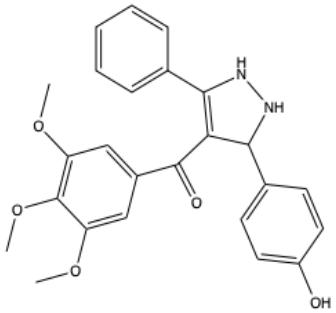


Figure 7: Structure of the first desired pyrazoline derivative.

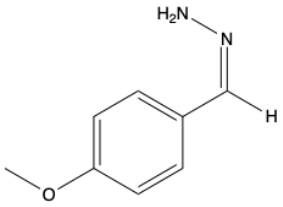


Figure 8: Structure of (1E)-(4-Methoxybenzylidene)hydrazone.

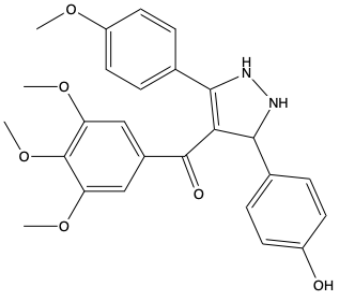


Figure 9: Structure of the second desired pyrazoline derivative.

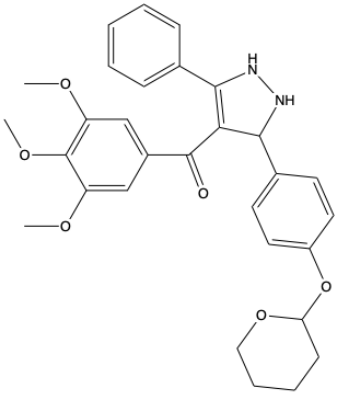


Figure 10: Structure of the third desired pyrazoline derivative.

3. Conclusion

In conclusion, the protection of 4-hydroxybenzaldehyde was proven successful. The subsequent chalcone synthesis from the protected benzaldehyde was also successful. The current synthesis of pyrazoline derivatives needs to be altered in order to avoid a reaction between only benzaldehyde and hydrazine. The reactions between chalcone, [DBU][Ac], and amine were unexpectedly found to result in the initial benzaldehyde.

4. Experimental

4.1. [DBU][Ac] (1,8-diazabicyclo[5.4.0]-undec7-en-8-ium acetate)

DBU (0.002 mol) was added to a 100 mL two-necked round bottom flask and cooled by an ice bath until the temperature reached 5 °C. Glacial acetic acid (0.002 mol) was slowly added to the flask. After the addition of acid, the flask was removed from the ice bath and refluxed on low heat with stirring for 26 hours. The reaction afforded a clear, colorless viscous liquid. The liquid was then dried in vacuo overnight to afford the desired ionic liquid.

4.2. (1E)-(4-Methoxybenzylidene)hydrazone

To a 25 mL round bottom flask, p-anisaldehyde (0.2 mL, 0.0016 mol), hydrazine monohydrate (0.08 mL, 0.0016 mol), and 1 drop [DBU][Ac] was added. The reaction was stirred at room temperature for 23.5 hours. TLC confirmed the completion of the reaction. The reaction was washed with 1M hydrochloric acid (3 x 20 mL) and saturated sodium bicarbonate (3 x 20 mL). The organic layer was dried with anhydrous sodium sulfate, vacuum filtered, and concentrated by vacuum evaporation to afford a yellow solid. ^{1}H-NMR revealed that the reaction produced the desired product (0.23 g, 95% yield).
^{1}H NMR ($CDCl_3$): 3.89 ppm (d), 6.98 ppm (d), 7.80 ppm (d), 8.63 ppm (s).

4.3. Protection of 4-hydroxybenzaldehyde (4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde)

To a 250 mL 2 necked round bottom flask, 4-hydroxybenzaldehyde (2.58 g, 0.0212 mol), pyridinium p-toluenesulfonate (0.798 g, 0.0032 mol) and dichloromethane (100 mL, 1.56 mol) was added along with a magnetic stir bar. After all reagents were dissolved, DHP (11.5 mL, 0.126 mol) was added dropwise by syringe. The flask was covered with aluminum foil and left to stir at room temperature overnight. TLC indicated that starting benzaldehyde was still present, so an additional amount of DHP was added (2.25 mL, 0.025 mol) and the reaction was left to keep stirring overnight. The resulting reaction was concentrated under reduced pressure. The concentrated reaction mixture was washed with 100 mL ddH_2O and ethyl acetate (2 x 50 mL). The resulting organic layers were combined and washed with saturated K_2CO_3 until the aqueous layer was colorless (approximately 5 x 30 mL). The resulting organic layers were combined, dried with anhydrous Na_2SO_4, vacuum filtered, and concentrated by vacuum evaporation, affording a pale orange liquid (4.05 g, 93% yield).
^{1}H NMR ($CDCl_3$): 1.50 ppm-1.99 ppm (m), 3.60 ppm (t), 3.79 ppm (t), 5.50 ppm (s), 7.10 ppm (d), 7.79 ppm (d), 9.84 ppm (s).

4.4. Chalcone from (4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde)

In a 100 mL round bottom flask, 3,4,5-trimethoxyacetophenone (0.42 g, 0.0020 mol) was dissolved in methanol (23 mL, 0.57 mol). While stirring, 6M NaOH (5.4 mL, 0.162 mol) was added. After one hour, the protected benzaldehyde (0.41 g, 0.002 mol) was dissolved in methanol (~4 mL, 0.099 mol) and added to the reaction mixture. Upon addition of benzaldehyde, the reaction was then heated to reflux and turned slightly yellow. The reaction was left to stir overnight on heat. The next day, the reaction mixture had turned orange, TLC suggested the reaction had gone mostly to completion, and was taken off heat. After the reaction had cooled to rt, ice was added while stirring, then placed in the refrigerator overnight. The contents of the flask were then vacuum filtered, affording a dark orange, gummy solid (0.40 g, 64% yield).
^{1}H NMR ($CDCl_3$): 1.59-1.90 ppm (m), 3.49 ppm (s), 3.91 ppm (t), 5.50 ppm (s), 7.10 ppm (d), 7.25 ppm (d), 7.35 ppm (d), 7.60 ppm (d), 7.79 ppm (d).

5. Acknowledgements

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6. References

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