Flavylium-Inspired Catalysts for Chromenone Functionalization

A Major Qualifying Project Proposal submitted to the Faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the degree of Bachelor of Science

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Report Submitted to:

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Abstract

Through the identification and modification of naturally occurring flavone compounds into usable photocatalysts, chromenones can be functionalized to be usable components for the overall synthesis of phomoxanthone A. This project centers the process of modification of three flavones from natural sources and a study of the scope of functionalized chromenones. Naturally occurring flavonoids are resistant to oxidation, have excellent electron delocalization, and occur in instances where they must be sensitive to light. All of these characteristics define an effective photocatalyst and when possessed to this degree all at once, beg further investigation on the nature and potential of these compounds. The basis of this research is the ability to modify these natural products for the specific purpose of chromenone functionalization along with the trials and modifications of flavonoid based photocatalysts in the study of chromenone analogs.

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Background

Natural Products

At the moment, one of the most researched fields in medicinal chemistry is the study and identification of naturally occurring compounds as a therapeutic method. These natural products (NPs) are a part of a vast array of different molecules with a variety of functions including anti-cancer, antibacterial, antifungal, antiparasitic, insecticides, and herbicides. The different fields that are able to use NPs are in agriculture, human, and veterinary medicine, to name a couple. Since the discovery of penicillin in 1928, over 23,000 NPs have been discovered and utilized.[1] This initial discovery led to large efforts to find and characterize NPs all around the world including the United States of America, Japan, and many countries in Europe. One of the most intriguing aspects of NPs is their wide variety in complexity and size. This has led to interest in all different fields in the sciences. The complex stereo-centers from these NPs were of particular interest to synthetic chemists attempting to be able to artificially synthesize the products. For biochemists and microbiologists the interests lie in the biosynthesis and discovery of new organisms that could contain molecules of interest. The need for large-scale production would need the work of chemical engineers and the list goes on and on of the many scientists intrigued in the world of NPs.

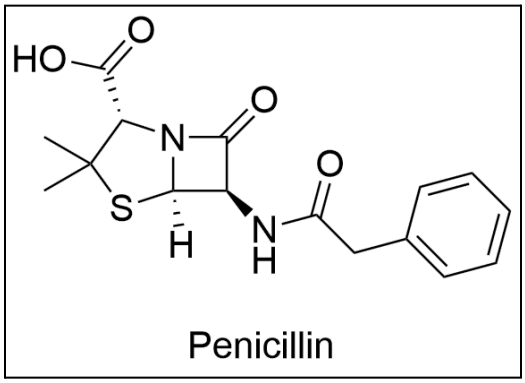


Figure 1: Penicillin

There are various different discovery methods that have been used in the search of NPs. Some of these methods include phenotypic screening, target-based approaches, and genomic-based studies. The phenotypic screening uses a specific set of cell lines that can be tested, such as a bacterial cell line, where the possible NP of interest can be added to and easily be tested for effectiveness, like an bactericidal antibiotic. Target-based approaches are based on having some knowledge of the target and mechanism of action of the NP. This approach is much more specific than phenotypic and can lead to more specific results. The genomics-based studies have become available more recently due to the ability to sequence the genomes of most organisms. It utilizes the sequences to look selectively for secondary metabolite gene clusters (SMGCs) which are segments of DNA that produce many NPs of interest. In many cases, the organisms contain some SMGCs, but they don't express the pathways or proteins that would form the NPs. With increasing knowledge and technology, scientists are able to induce these pathways to express the potential NPs.

Xanthones as Natural Product Therapeutics

Xanthones are natural products that were originally discovered in plants. From 2012 to 2019, a total of 1225 xanthones had been discovered from over 20 different plant families.[2] Xanthones have demonstrated cytotoxic effects that can be possibly used as anti-cancer agents. As is the case with many NPs, it is difficult to obtain large amounts of the NP from the plants they naturally occur in since they are only available in low concentrations. This struggle has led to the introduction of organic chemists to work on total synthesis projects that would be cheaper and hopefully increase the yield of the xanthone and its analogues. There have been multiple different methodologies used to synthesize xanthones including starting with benzophenone, diaryl ether, and chromen-4-one.[2]

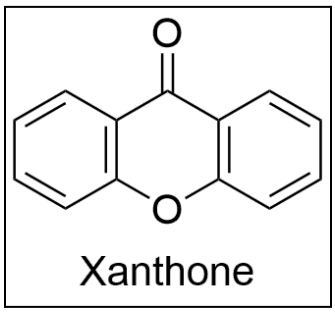


Figure 2: Xanthone

Studies have been done recently to look at the mechanism of action and the effects that xanthones have on cells, which have shown that they have anti-cancer and anti-inflammatory effects with low toxicity in normal cells. One study demonstrated that α-mangostin, a xanthone derivative, inhibited YD-15 tongue mucoepidermoid carcinoma cells through the induction of apoptosis.[3] In another study by Kristanawon et. al, they found similar results using α-mangostin which induced apoptosis through the HER2/PI3K/Akt and MAPK signaling pathways.[4]

Another important xanthone related NP is the homodimer phomoxanthone A. It was isolated and discovered by Isaka et al. in fungi from the *Phomopsis* genus. [5] The *Phomopsis* genus is known to be rich in secondary metabolites such as phomopsichalasin and phomopsin A, which are a cytochalasin and mycotoxin respectively. While phomoxanthone A was discovered while searching for antimalarials, it demonstrated strong anticancer properties. A special aspect of phomoxanthone A is that it is able to induce apoptosis even in cisplatin resistant cancers.[6] Platinum compounds are the first line of defense against many cancers, but recently many cancers have shown resistance. This is where phomoxanthone A comes in. Phomoxanthone A was shown to have cytotoxic effects on cancer cell lines, but was not shown to affect normal human lymphocytes.[7] Pavão et al. demonstrated that phomoxanthone A showed no cytotoxicity, genotoxicity, or mutagenicity on normal human lymphocytes, but exerted all on HL60 cells. One of the drawbacks of being a natural product is that there are many difficulties in the synthesis, especially in the under researched area of chromanones and tetrahydroxanthones.[8]

Flavonoids

Another group of natural products that have shown strong antioxidant properties are plant-based flavonoids.[9] They are abundant in many plants and seeds and are responsible for many characteristics such as color. These NPs function in the native species as cell growth regulators, protecting against stressors, and UV filters. Our NPs arise in plant cells in the highest concentration when there are conditions of low temperature and scarce water, where photosynthesis is still promoted due to ample light yet the cell is unable to metabolize this energy due to lack of other resources to therefore minimize the damage this would otherwise cause on the organism (Figure 3).[10] These metabolic flavonoids exist most often in higher and more developed species of plants and the most common categories are flavonols, anthocyanins and polyamides. These polyamides, though not a focus of this work, are chemically similar and exist most plentifully in the shells of seeds. The metabolic branch pathways that these compounds are related to in plant cells are the flavonol, anthocyanin, polyamide, isoflavone, orange ketone, and tannin cycle. This array of pathways is not only connected through byproducts and starting materials, but also through similar mechanisms of action.

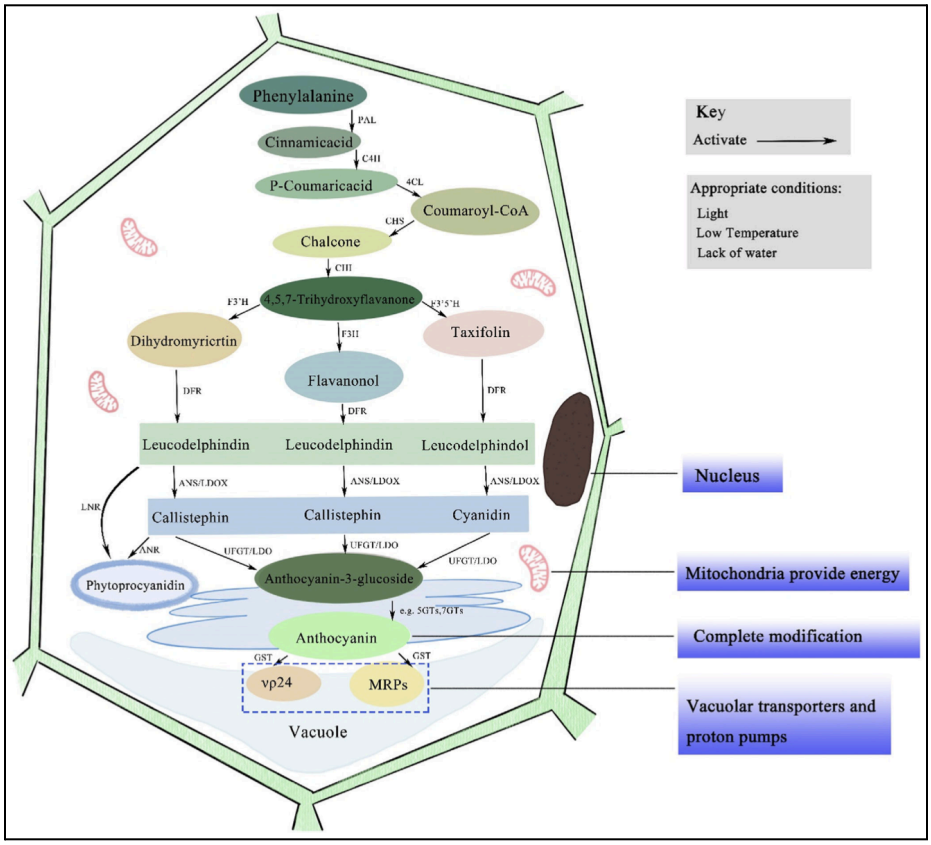


Figure 3: Flavonoid metabolites in a plant cell. The transformation of metabolites through the web of available pathways in a higher species plant cell, specifically the presence of the

secondary metabolite- Flavonol. Within the pathways of this figure are flavonoids like flavonols, anthocyanins and polyamides[10].

In humans, flavonoids have shown a large range of health benefits including antidiabetic, antibacterial, and antiviral effects. However, the presence of hydroxyl groups on many flavonoids affect humans' biological activity.[11] The strong antioxidant properties of flavonoids can make them a possible reagent for different chemical synthesis reactions as well as a player in the cell growth cycle and vascularization therefore the cardiovascular system (Figure 4).

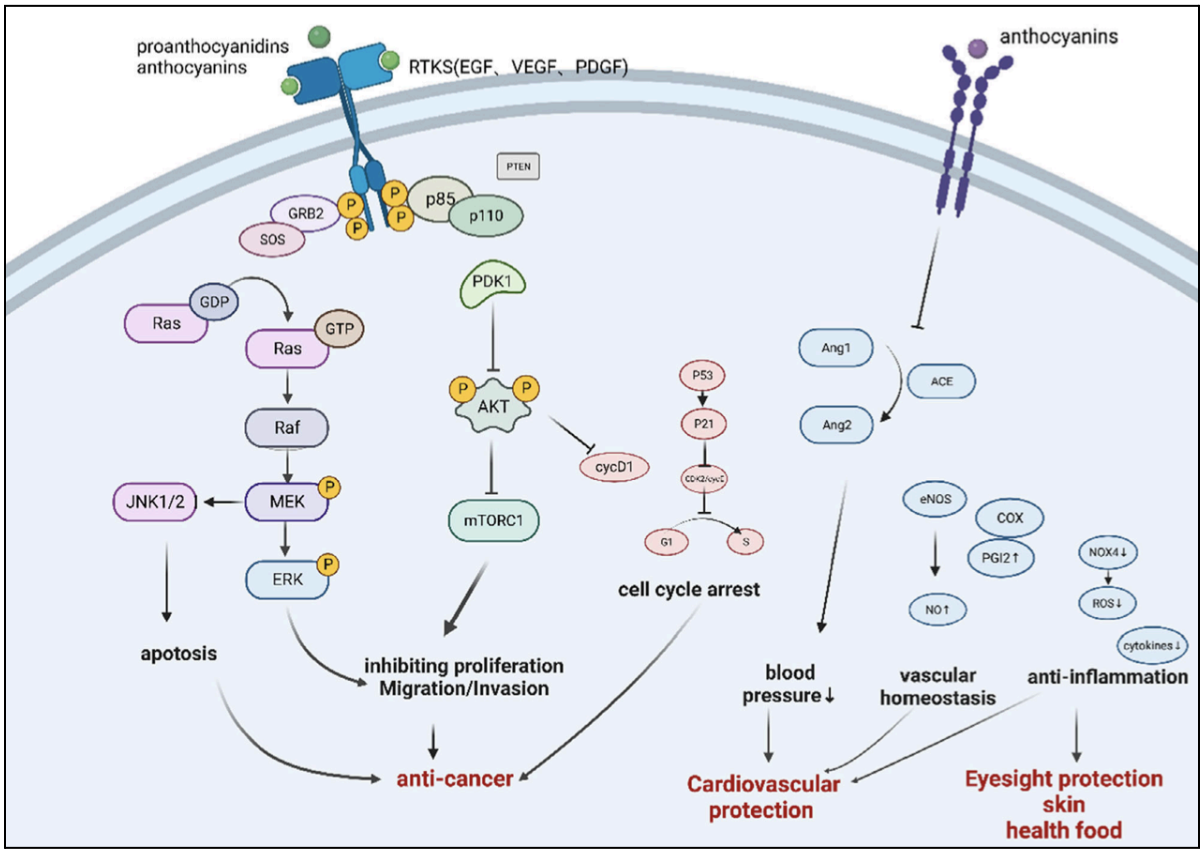


Figure 4: Action of anthocyanin and proanthocyanidin in a human cell. A potential mechanism of action of anthocyanins and proanthocyanidins on a human cell, outlining the cardiovascular protection and anticancer properties of these compounds [10].

With a higher resistance to oxidation, these compounds are able to be reliable protectors of cells against the damage of free radicals. By doing this, the cell experiences an array of benefits such as increased elasticity of blood vessels, improved overall circulatory system, decrease allergies and inflammation, and improve joint flexibility.[12] More generally, these anthocyanins help the cell absorb and use vitamin C and E, maintain collagen synthesis, and inhibit tyrosinase to protect against UV radiation and other types of sun damage.[13] These

compounds are not only effective in combating the harmful obstacles our body may face, but have been active factors in the history of holistic and scientific health and cosmetic upkeep.

Current Mattson Group Work and Goals

The current work in the Mattson Group is centered around the discovery, synthesis, and functionalization of different NPs. The current goal is the synthesis of phomoxanthone A using other NPs as precursors. This process is being done through the functionalization of differently substituted chromenones in order to form one subunit of the phomoxanthone homodimer and then be able to couple the subunits to form phomoxanthone A analogs. The current methodology uses flavonoid NPs as a photocatalyst in the functionalization of chromenones.

Results and Discussion

Sourcing and Isolation of Flavonoids

In search of naturally occurring flavylium compounds that will serve as photocatalysts in the functionalization of chromenones, the select bioactive characteristics that flagged a deeper look were antibacterial, antioxidant, and anti-inflammatory properties. The capacity to be this way is achieved through high power oxidation and these molecules and in some scenarios, when coupled with photoreactivity can fulfill the specific yet overall undiscovered mechanism employed in chromenone functionalization. The next lens forced us only to consider molecules that were easily subject to this specific functionalization, most molecules were deemed inaccessible due to highly substituted skeletons. This entailed crowding of hydroxy and methyl groups that would interfere in the subsequent Grignard and benzylation. The core skeleton of the flavone compound pictured in Figure 5, along with three of the six classes of flavonoids. The flavonoids that were chosen for functionalization were transformed into anthocyanin analogs and then into the final photocatalyst product that would be purified through a series of solvent systems tests and preparative TLC plates. The spots on the preparative TLC plates were extracted and then analyzed via H-NMR.

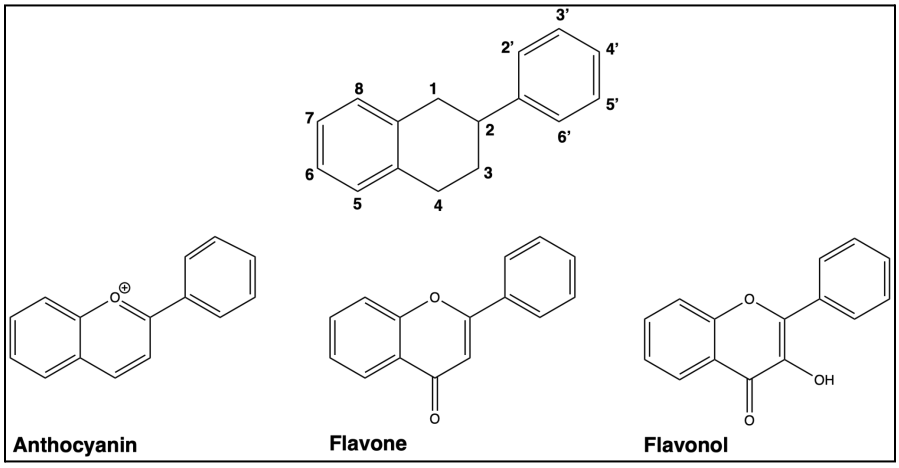


Figure 5: Flavonoids and the three main studied classes.

Chrysin and Quercetin were the two naturally sourced flavonoids, a flavone and a flavonol respectively that can be seen in Figure 2, can be found in honey, onion, fruits, and other plants. A notable factor to this study is the fact that these compounds exist readily in a healthy diet and therefore hold more relevance to us, especially in sourcing readily available compounds as tools in drug discovery. Both of these compounds were synthesized, Chrysin through a partnership with Worcester State University and within our lab to be held in experimentation while isolation efforts on naturally occurring sources could be held separately.

Isolation of naturally occurring quercetin was performed on locally sourced commercial yellow onions as this variety had been reported to have the highest levels, as well as overall

across fruits, vegetables, and herbs when also considered with affordability and efficiency when compared to the runner up that is dill.[14] This process was done by peeling the skin off approximately 15 onions at a time to obtain increments of 20-25 mL at a time for extraction. The skins were dried and then refluxed in enough ethyl acetate to completely submerge for 24 hours, solution was filtered, dried, weighed, and then put through a series of purification processes to reach pure quercetin in a chemically conservative way. Through analysis via TLC prior and following each purification step, minimal difference was made in nearing a pure solution. Recrystallization in ethyl acetate, an ethyl acetate and hexane solvent system, and ethanol were done, H-NMR analysis of the prior supports the conclusion of a still crude final product that was unable to be purified further within the time restriction.

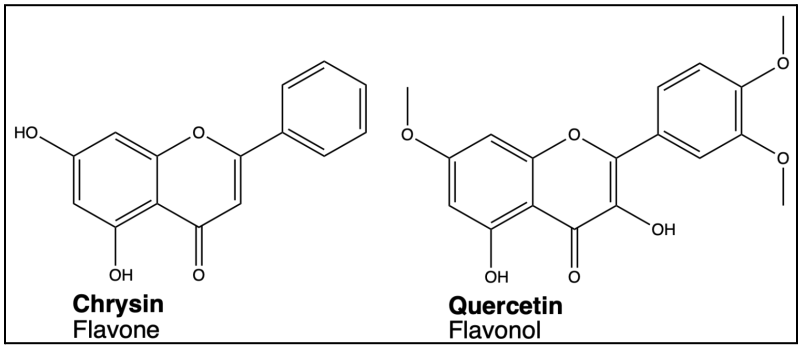


Figure 6: The two base flavonoids used in this study.

There were no isolation attempts on chrysin, however the original influence of this compound with the trajectory of the project was started by the covalent coupling of it in aging wine for stabilizing these water sensitive compounds. While identifying the naturally occurring sources of this pigment, both in wine and in the largest quantity found in nature being wild honey of the forest variety. This is indicated through the deep brown and almost maroon color of this type of honey and would have been the source of isolation through the purpose of this project as there are fewer chemically similar pollutants, fewer different compounds in general (approximately 180 compared to approximately 800) in a 1:10 ratio between the two substances, and is more cost effective to deliver the most efficient isolation in terms of chemical economy, cost, and difficulty in attainability. The chrysin that was sourced was microwave made and advised to have pollutants present, this was identified as pyridine through H-NMR spectra and was recrystallized in minimal ethyl acetate at above 85% yield.

Functionalization of Catalysts

In the functionalization of chrysin, the development in synthesis of the desired photocatalyst was inspired by the natural process that occurs in most aged red wines in the maceration stage as a color preservation mechanism to protect the flavylium chromophore from hydration.[15] This covalent mechanism turns water sensitive oenin, an anthocyanin, into a modified color-stable compound that adopts either a more blue or orange tone and becomes much more resistant to oxidation and hydration. This newfound resistance is due to the

conjugated double bond and a change in the substitution pattern that allows for more delocalization of electrons. This mechanism was adapted into a two step synthesis replicated on both flavonoids in order to formulate, analyze and test.The final process that was used to formulate these photocatalysts is described in Figure 7.

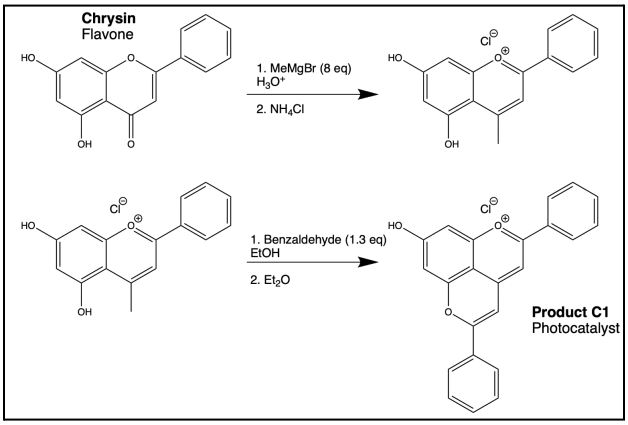


Figure 7a: The procedure that was employed for the formation of a chrysin inspired Pyranoflacylium chromophore referred to as Product C1.

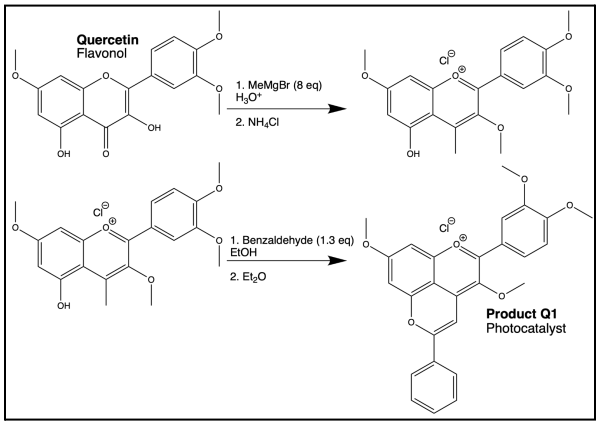


Figure 7b: The procedure that was employed for the formation of a quercetin inspired pyranoflavylium chromophore referred to as Product Q1.

The procedure that was formulated consisted of a grignard under a low heat reflux within the atmosphere of an N_2 balloon for 2 hours followed by being quenched in excess NH_4Cl for 2 hours before being collected off and dried via vacuum filtration which then gave the product in pure forms. In the reaction scheme shown above of chrysin (Figure 7a) the initial reaction was initially performed with 0.08 mmol of the starting material and 0.56 mmol, 7 molar equivalents, of the grignard reagent. Within these trials, the yield of the product averaged 60% which led to further modification of this procedure. In the first trials performed with 8 molar equivalents of the grignard reagent, the crude yield jumped significantly to average at 94% which set the standard for this and the parallel reaction (Figure 7b). The yield for this parallel reaction, the grignard of Quercetin(Figure 7b), for the second reaction averaged to be 54%. In both scenarios, H-NMRs first were being taken on $CDCl_3$ but characteristic peaks were not present and this was then later confirmed to interact negatively with the product. Despite knowing that this anthocyanin would be more stable than the mother compound especially in more acidic-like conditions provided by the deuterated chloroform, this was unexpected and D-DMSO was attempted next knowing that due to it being a much more inert solvent in the face of organic compounds despite being more sensitive to water. These H-NMR reads were much more clear and comprehensive in containing the appropriate peaks indicative of the desired product from the grignard reaction. With still the prevalence of contaminants in this system, TLC solvents were attempted to separate out the components of the previous single product spot despite this spot

serving an R_f of below 0.8 indicating acceptable result. Specifically, this value fell at 0.73 for the chrysin product and 0.64 for the quercetin product. This fact remained constant for the product from the grignard on both initial reactions. Through various systems, additional spots were unable to be pulled out and identified, therefore indicating further trials needed to be done or a more inert H-NMR solvent required trial.

In the development of the second step, the natural parallel is the flavilium-(4→8)-flavan chromophore formation through the oxidative condensation of a flavylium salt with catechin, and in a wider scope varied forms of general coupling that gives rise to new product further fortifying the stability of these naturally occurring pigments. This metabolic function of plants and plant products has allowed for the presence and stabilization of large compounds that serve excellently as tools for electron delocalization. This reaction was run with half the amount of starting material as the initial step, leading to an overall lower yield due to a potential higher ratio of pollutants to product and with a less than theoretical yield, leading to even less product. This reaction was run continuously with a 1.3 molar equivalent to benzaldehyde and the time in which this reaction was run was the factor that was varied in order to increase yield. This slight time study consisted of following the recommended time for reaction and then assessing product yield if this time were to be doubled. However, no significant conclusions were able to be drawn from this specific series of reactions as this myriad of factors had resulted in key methyl groups of the quercetin product being absent and in the case of the chrysin product the pollutants present in the sample upon every run rendered the product undetectable. However in these experiments, the color modification of chrysin resulted in the original brown/orange color to transform into a deep maroon and the quercetin that was an original yellow/white product to become a vibrant violet. Pictured below, the maroon color that developed was indicative of product as outlined by the most influential procedure for this process, and the ladder aligned with the increased blue tones of a fortified pyranoflavylium chromophore which explained the purple color. Pictured below in Diagram 1 and 2 are the product and filtrates within the final step of this two step synthesis in different phases.

Diagram 1: The final product formation of the chrysin based pyranoflavylium chromophore. As seen drying under vacuum filtration, the maroon color of the mother liquor similar to the once tar-like product after washing with EtOH.

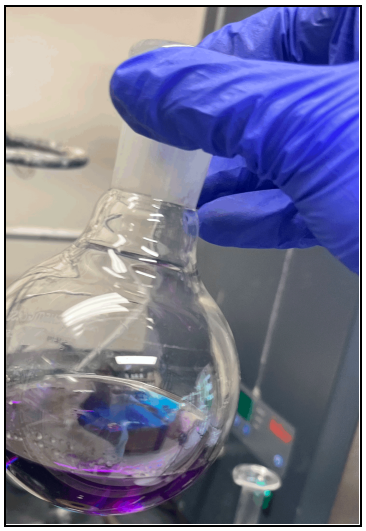


Diagram 2: The final product of the violet quercetin based pyranoflavylium chromophore. Product has been dissolved in EtOAc from the filter for maximum recovery.

Purification was necessary after the formation of both final products, and this was done through a series of washings and recrystallizations in ethanol. The first round of cleaning the product was performed via washing the filtered product in chilled ethanol (3 x 5 mL) before drying completely to rinse out benzaldehyde, starting material, and byproducts. The expected yield of this reaction as outlined in the original procedure fluctuated between 50-78% therefore leaving a maximum of 0.0323 mmol of product in either scenario totaling at 0.1096-0.1431 grams at the highest. Though this was not observed, experimentation was replicated numerous times in order to diminish the likelihood of human-introduced error as well as to fluctuate the reaction time in order to find a setting that gave the clearest presentation of the product at the highest yield.

Chromenone Functionalization by Photocatalysts

The general reaction scheme used in the functionalization of chromones is seen in Figure 8. This reaction used a chromenone starting material, photocatalyst, and tetrahydrofuran(THF) as the reactant in order to form the chromanone product. The reaction uses 1 EQ of starting material, 30% of TMSOTf, 20% photocatalyst (Flavone), and 5 EQ of the reactant. Dichloromethane is used as the solvent and totals 3 mL in the reaction vial.

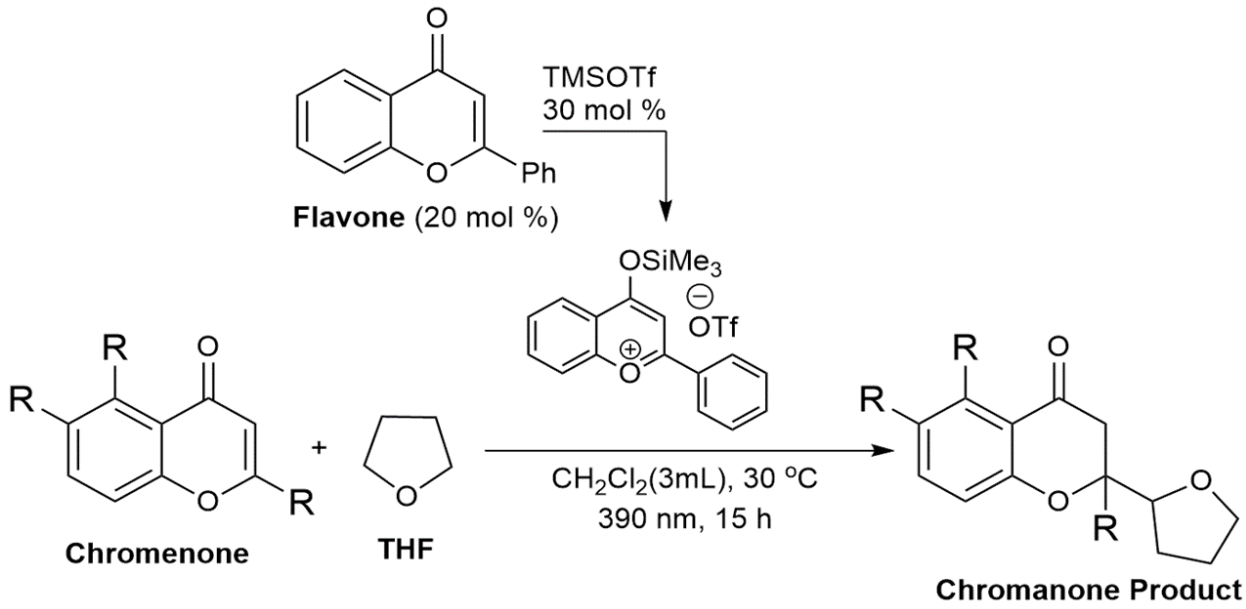


Figure 8: General reaction scheme for chromenone functionalization.

The functionalization of chromenones started with the synthesis of Flavone in a three-step using the reaction mechanism in Figure 9. The reaction starts with o-hydroxyacetophenone and benzoyl chloride to form o-benzoyloxyacetophenone. Through the addition of potassium hydroxide, it forms o-hydroxydibenzoylmethane. The addition of sulphuric acid, glacial acetic acid, and heat forms Flavone. The overall yield ranges from 50-68%.

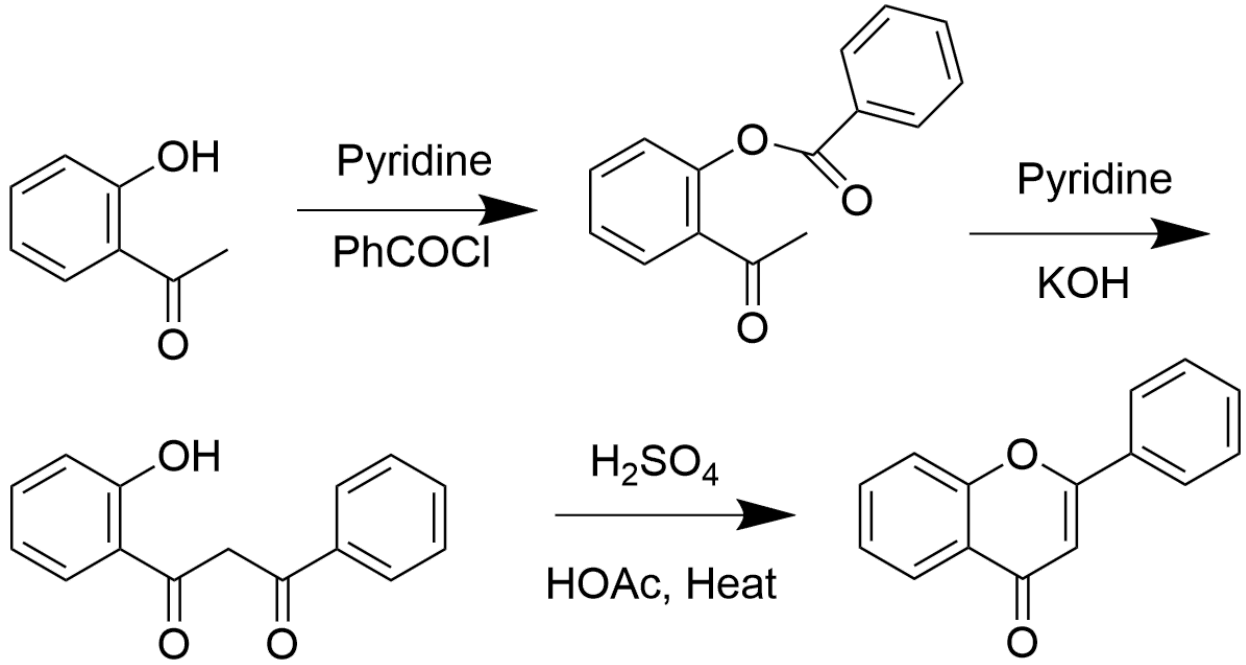
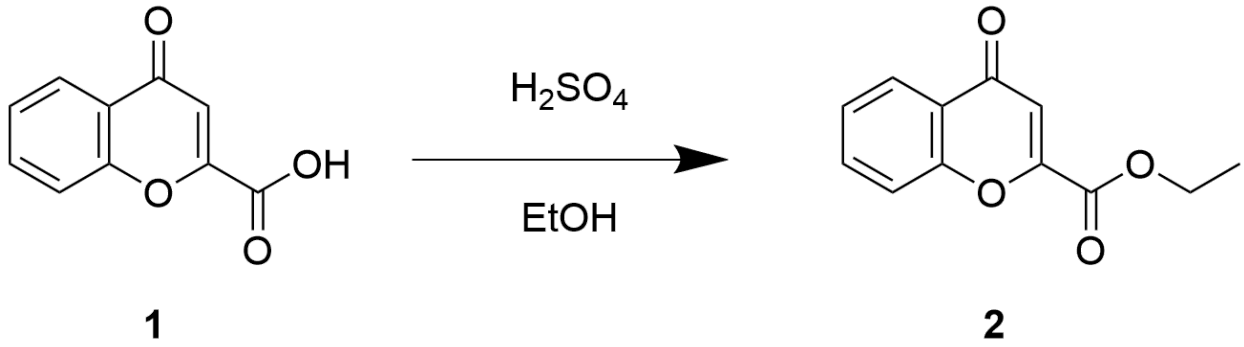


Figure 9: The reaction scheme used to synthesize Flavone from o-hydroxyacetophenone.

Although most of the starting materials were readily available in the lab, synthesis was needed to prepare **2** in the reaction seen in Figure 10. The general method for synthesis follows Procedure C, a Fischer Esterification. This reaction used 15.6 mmol of **1** and 0.33 mmol of sulphuric acid. The identification of **2** was confirmed using H-NMR.



1 **2**

Figure 10: Reaction scheme for Fischer Esterification to form compound 2 from compound 1.

Starting Material and Product Scope

For the starting material scope, the studies were done to contain a variety of different substitutions on the base chromone. These substitutions were present at the 2, 6, or 7 position of chromone as seen in Figure 8. All of the substitutions are electron withdrawing groups, which allows for comparisons between the different substitutions. **2** and **3** both contain either a methyl ester or ethyl ester at the 2 position respectively. **6** and **7** contain different halogens at the 6 position, with **6** having fluorine and **7** containing bromine. **5** has a hydroxyl group substituted at the 7 position and **4** is unsubstituted. **4** was the only reaction ran with 1,2-bis(trimethylsilyloxy)ethane. The following figures contain all the starting materials followed by all of their products.

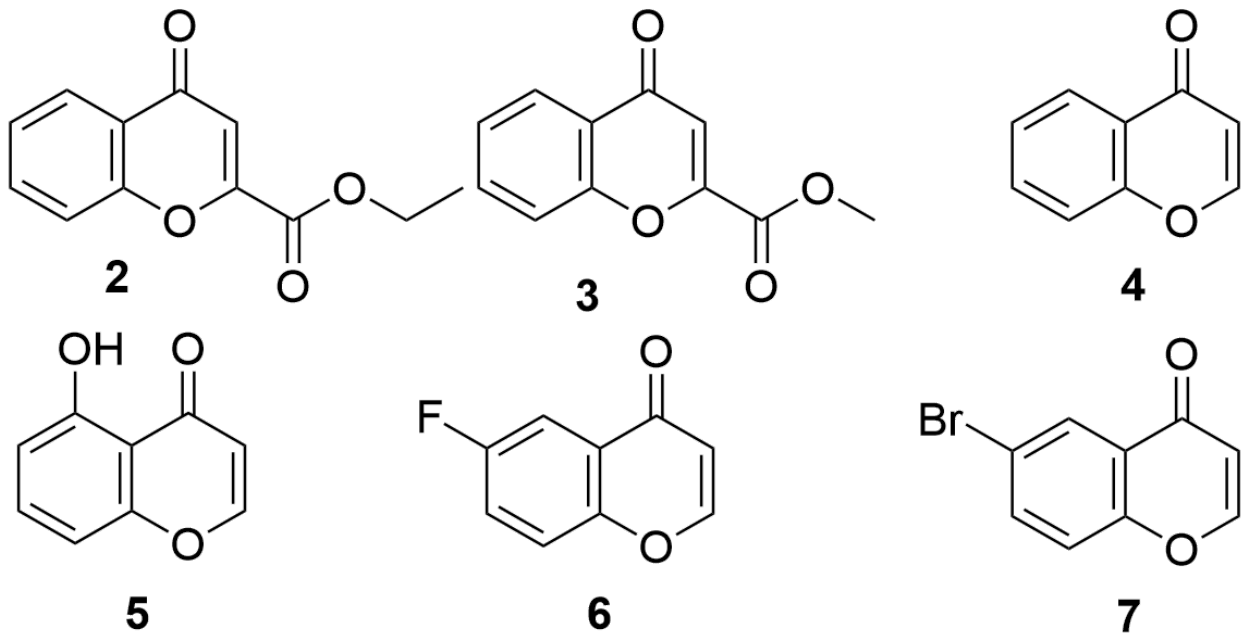


Figure 11: List of all the starting material used in the functionalization of chromenones.

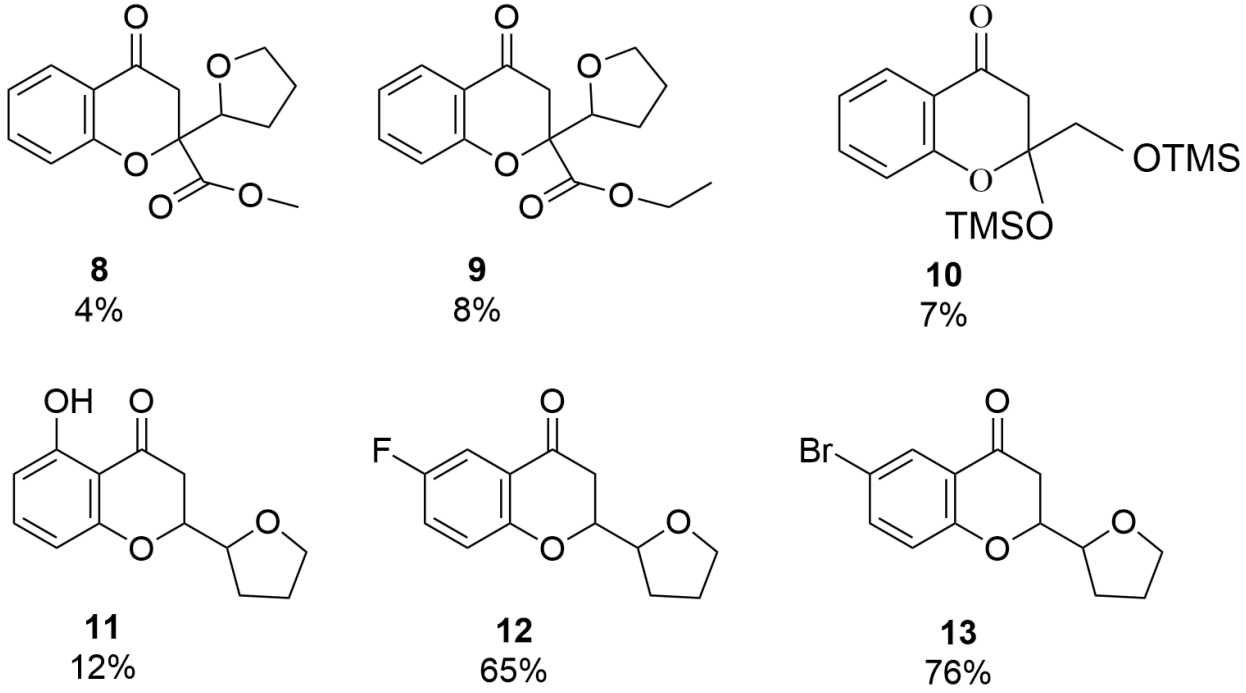


Figure 12: List of all the products produced in the functionalization of chromenones with H-NMR yield.

Purification, Isolation, Determination of Diastereomers

In order to separate and characterize the different diastereomers that were formed in the reaction in Figure 8, the excess solvent was evaporated using the rotary evaporator(rotovap) and put under the high-vacuum until as much solvent was removed as possible. The remaining sample was then resuspended in ethyl acetate. Thin-layer chromatography plates were then split in half and then the samples were added to the plate horizontally. The solvent that was used was 80:20 Hexanes:ethyl acetate for approximately 20 minutes. The plates were then studied using short-wave UV light. The 2 diastereomers appear as purple-blue bands separated around the middle of the plate. These bands are separately added to pasteur pipettes where the samples are eluted using ethyl acetate. The solvent was then evaporated using the rotovap and high-vacuumed. They were then analyzed using H-NMR.

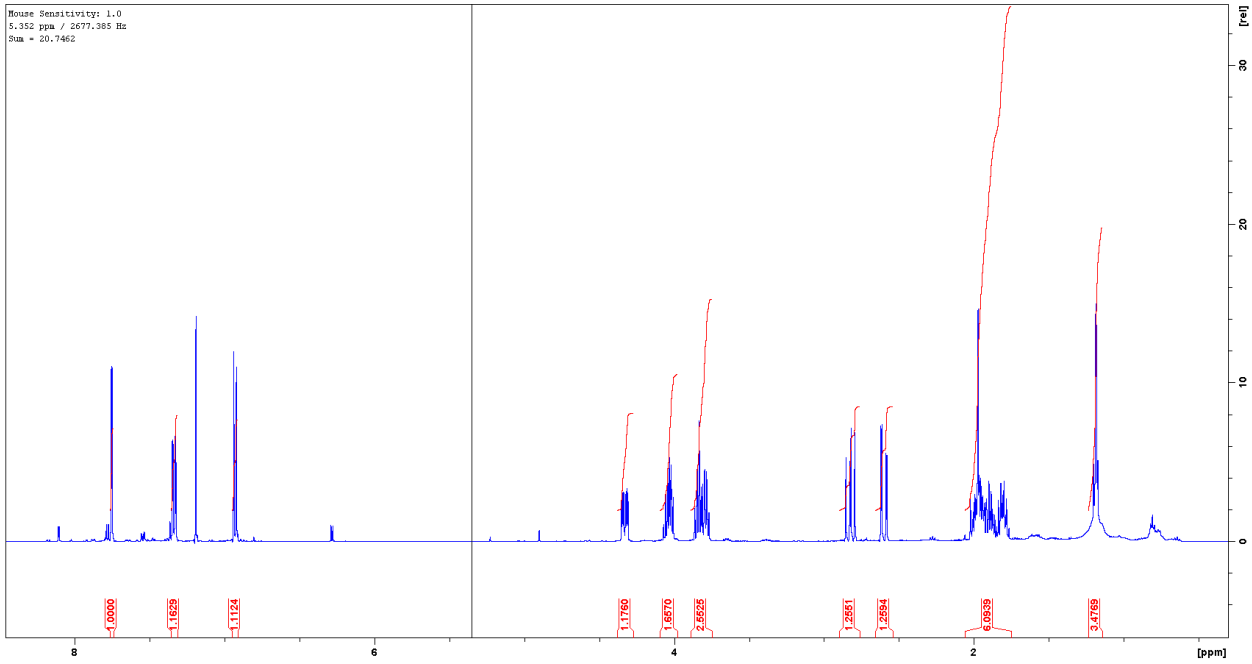


*Figure 13: H-NMR of the bottom spot of **12**. Two integrations taken between 2.5 and 3 ppm display the 2 doublet of doublets that identify a diastereomer.*

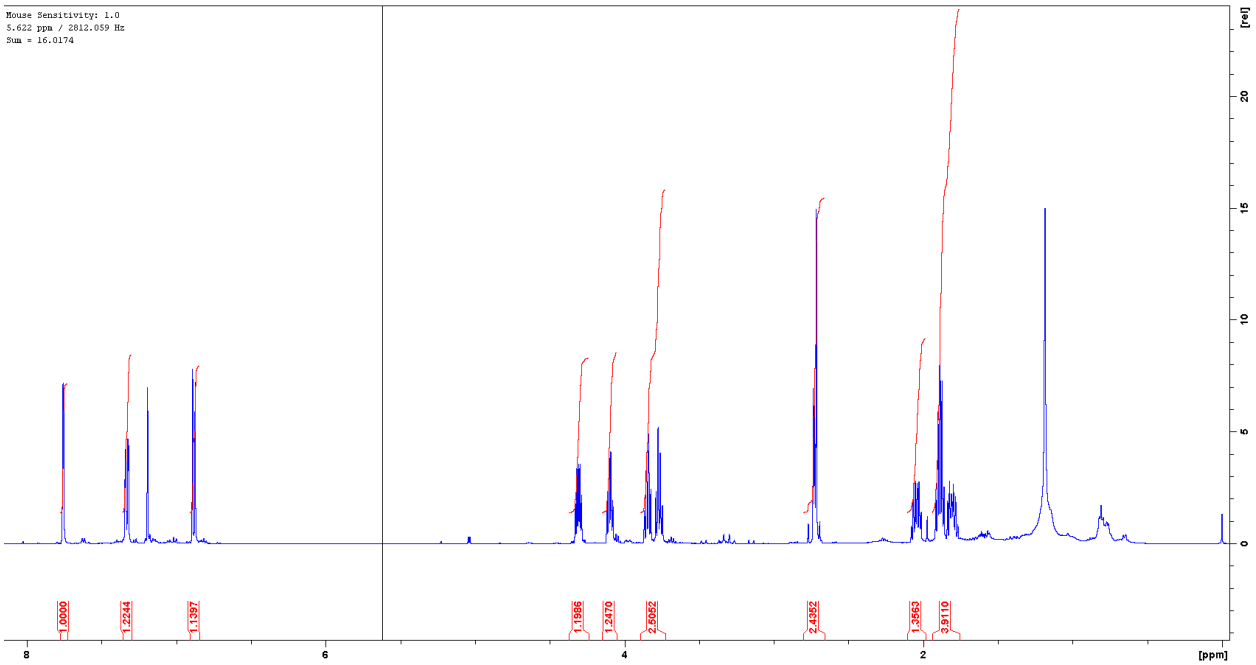


*Figure 14: H-NMR of the top spot of **12**. Two integrations taken between 2.5 and 3 ppm display the single quartet that identifies a diastereomer.*

These H-NMRs are from further isolation of the reaction mix that contained **12**. In Figure 13 , between 2.5 and 3 ppm, there are two separate peaks whereas in Figure 14, there is only 1 peak between 2.5 and 3 ppm. This is indicative that the two diastereomers were successfully separated.

In all cases, the samples were never made completely pure. In general, one of the diastereomer products tends to get stuck with the starting material on the TLC. In order to combat this, some plates were run vertically with the same solvent system of 80:20 hexanes: ethyl acetate. Even in this case, the product was still not able to separated completely from the starting material. We have attributed this partially due to some of the reactions producing low yields. When there are lower yields, it is much harder to separate the smaller amount of product with the much larger amount of starting material. In the H-NMR, we also found there to still be trace amounts of what we attribute to ethyl acetate from the elution of the samples from the TLC plate. We also identified some peaks that are attributed to leftover solvent in the NMR tubes including acetone and DCM.

At the moment, the exact structural differences between the diastereomers have not been elucidated, but work is being done in the Mattson Lab on identifying them. Although the structures themselves aren't known, the differences in NMR reads as mentioned above is still indicative of the presence of different diastereomers.

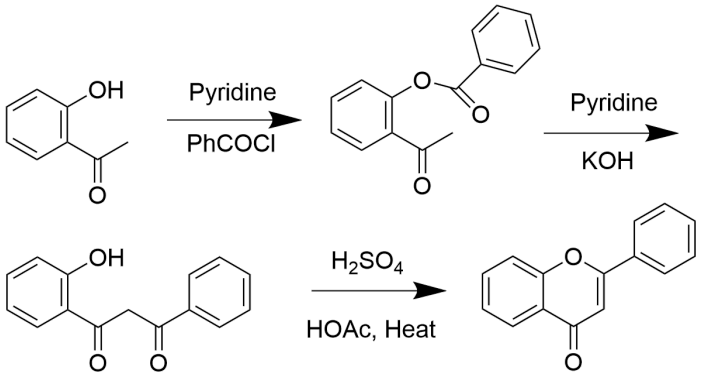
Conclusion

Though the two synthesized photocatalysts were unable to be tested in chromenone functionalization, the work resulted in the continuous findings of sustainable and more economically efficient methods for the commercial synthesis when considering phomoxanthone A. These compounds were synthesized in accordance to previously referenced procedures and observations aligned on all fronts of product formation. In addition to this, our study has demonstrated that chromenone functionalization through the use of the simplest flavonoid photocatalysts is a viable method. We found that ester substitutions at the 2 position, compounds **8** and **9**, yielded 4-8% product. This yield was significantly lower than was found for halogen substitutions at the 6 position,compounds **12** and **13**, which resulted between 65 and 76% yield. Due to low yields of 12%, one diastereomer of **11** could not be appropriately separated from starting material. The reaction to form **10** produced a 7% yield from the reaction with 1,2-bis(trimethylsilyloxy)ethane. Previous work in the Mattson lab has demonstrated that reacting **4** with THF instead of 1,2-bis(trimethylsilyloxy)ethane produces a 86% yield, which is significantly higher. This demonstrates the possibility that 1,2-bis(trimethylsilyloxy)ethane could be a less efficient reactant than THF, but further work will need to be done to determine if this is true.

Appendix A: Procedures

Procedure A:

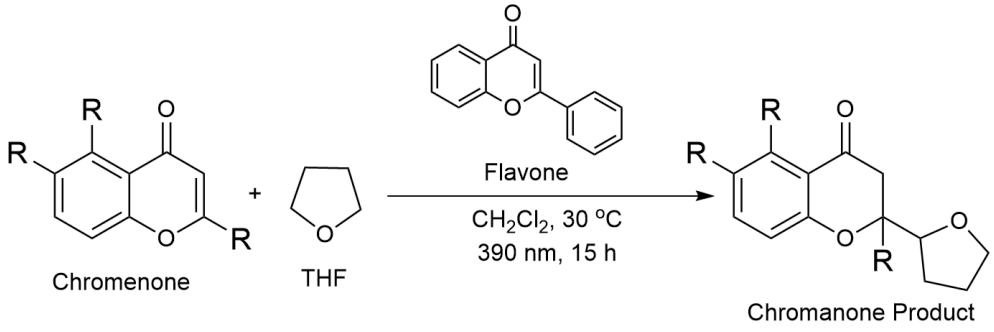
Flavone Synthesis:



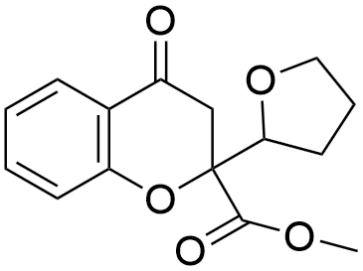
645 mg of acetophenone and 22 mL of pyridine to a beaker. 0.7 mL of benzyl chloride and let it stir, 1.6 g of KOH was added and continued stirring at 50 ℃. The next day mixture was quenched in water, filtered using a side arm erlenmeyer, and dried using vac. The next day, 161 **µ**L of sulphuric acid and 3.95 mL of acetic acid were added and it was stirred at 50 ℃. The next day, the mixture was mixed with ice water and stirred and filtered using side arm erlenmeyer and washed again with water.

Procedure B:

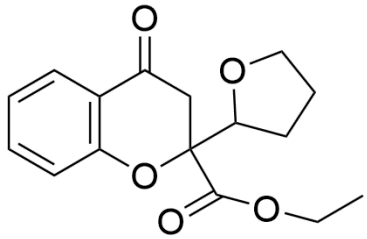
Chromanone Synthesis General Reaction:



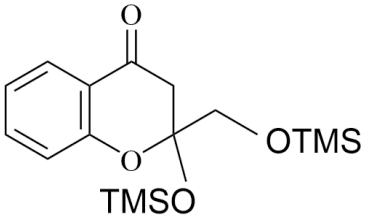
To a 1 dram vial, 0.2 mmol of starting material and 0.04 mmol of Flavone were added and then a vacuum was pulled. Using a 6 mL syringe, vacuumed dichloromethane was added to the vial. Then using a microneedle, 0.1 mmol of reactant and 0.06 mmol of TMSOTf were added separately to the vial. The vial was put on a small rack between two UV lights set at 390 nm, with a fan, and with a magnetic stirrer to run overnight.



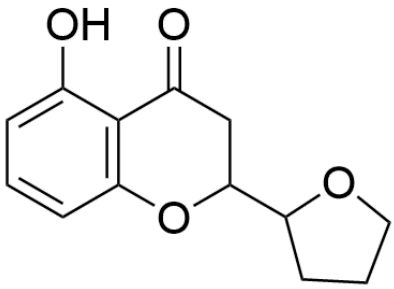
methyl 4-oxo-2-(tetrahydrofuran-2-yl)chromane-2-carboxylate: Prepared according to the procedure B, using **3** (40.8 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), THF (81.2 **µ**L, 0.1 mmol)



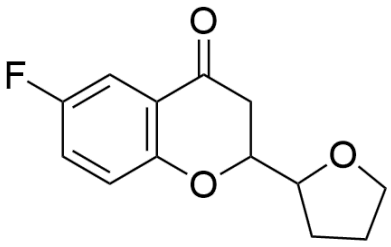
ethyl 4-oxo-2-(tetrahydrofuran-2-yl)chromane-2-carboxylate: Prepared according to the procedure B, using **2** (43.6 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), THF (81.2 **µ**L, 0.1 mmol)



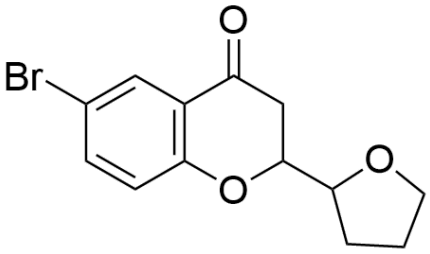
2-((trimethylsilyl)oxy)-2-(((trimethylsilyl)oxy)methyl) chroman-4-one:
Prepared according to the procedure B, using **4** (29.2 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), 1,2-bis(trimethylsilyloxy)ethane (245 **µ**L, 0.1 mmol)



5-hydroxy-2-(tetrahydrofuran-2-yl)chroman-4-one: Prepared according to the procedure B, using **11** (32.4 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), THF (81.2 **µ**L, 0.1 mmol)



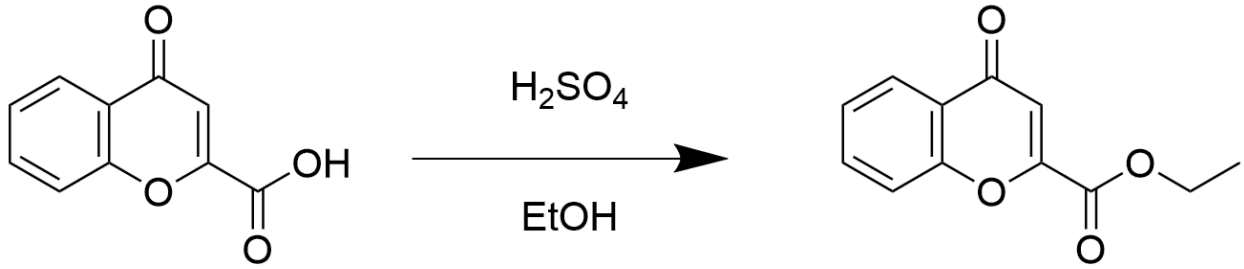
6-fluoro-2-(tetrahydrofuran-2-yl)chroman-4-one: Prepared according to the procedure B, using **12** (33.03 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), THF (81.2 **µ**L, 0.1 mmol)



6-bromo-2-(tetrahydrofuran-2-yl)chroman-4-one: Prepared according to the procedure B, using **13** (45.2 mg, 0.2 mmol), Flavone (9.1 mg, 0.04 mmol), TMSOTf (11 **µ**L, 0.06 mmol), THF (81.2 **µ**L, 0.1 mmol)

Procedure C:

Fischer Esterification:



1 **2**

In a 150 mL round bottom flask 15.6 mmol(3 g) of 4-oxo-4H-chromene-2-carboxylic acid(**1**) was mixed with 45 mL of ethanol and chilled in an ice bath. 0.33 mmol(18 mL) of H_2SO_4 was slowly added. The round bottom flask was put in 80 ℃ overnight. Next day the solution was poured into a 100 mL beaker in an ice bath. A filter and side arm erlenmeyer was used to filter out the solid from the solution. Sodium bicarbonate was added sparingly to quench any acid leftover. The solid was then scraped out into a 2 dram vial. Methanol was heated to 62 ℃ and added to the vial with solid while keeping the vial on heat to dissolve the solid. The product and methanol were put in a freezer until crystals formed. Once crystals formed, the solution was filtered using a filter and side arm erlenmeyer flask. Cold methanol was used to completely empty the vial.

Appendix B: Analytical Methods

Chromenone NMR Methods:

H-NMR Yield:

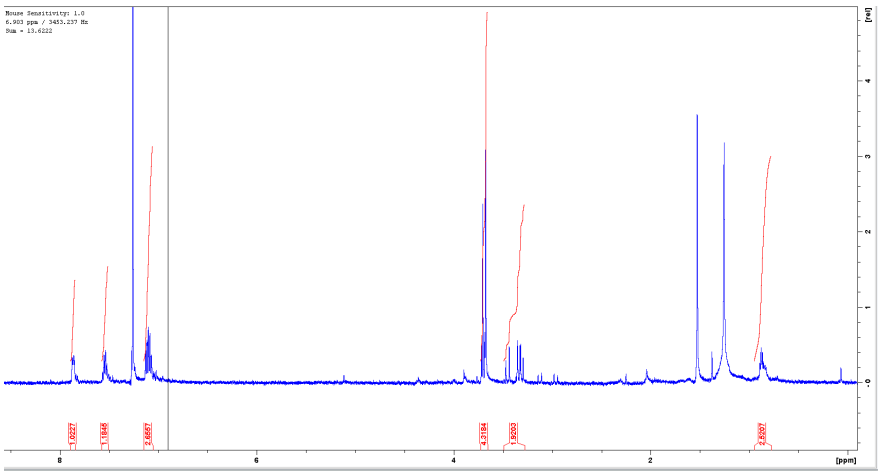
The H-NMRs that were used to find yields used deuterated chloroform ($CDCl_3$) for the solvent and 28 **µ**L of mesitylene and 5 **µ**L of dibromoethane were added for internal standards. In order to determine the yields, the mesitylene peak at 6.76 ppm was integrated and set to 3.00. Then the diastereomers peaks at 4.06 and 4.125 ppm were integrated and the resulting integrations were added together to achieve the yield.

Purified Diastereomer Identification:

The H-NMR used to determine the purity and contents of the chromanone diastereomers used deuterated chloroform ($CDCl_3$) with no internal standards. The determination of different diastereomers were investigated by looking between 2.5 ppm and 3.0 ppm. One diastereomer contains separate sets of doublets of doublets whereas the other contains a connected doublet of doublets.

Appendix C: H-NMR Spectra

8



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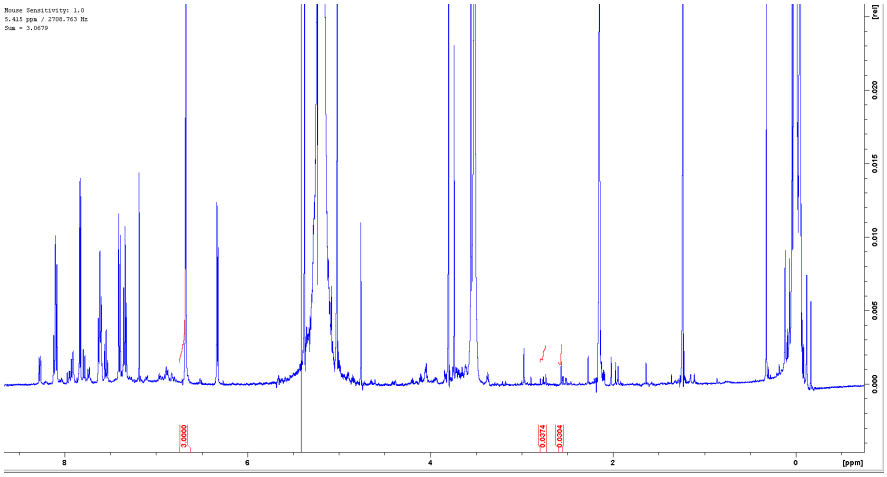

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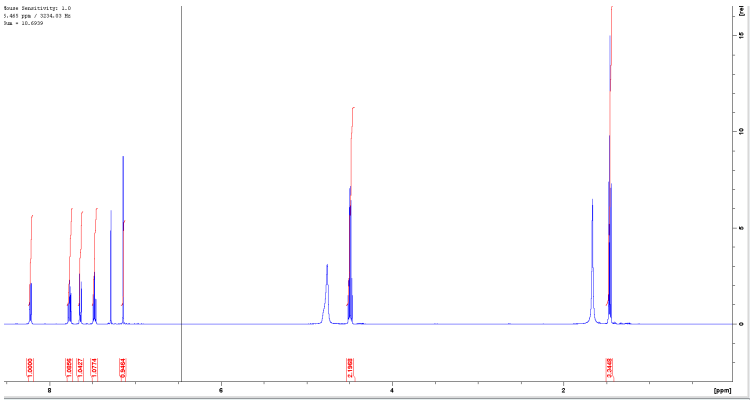
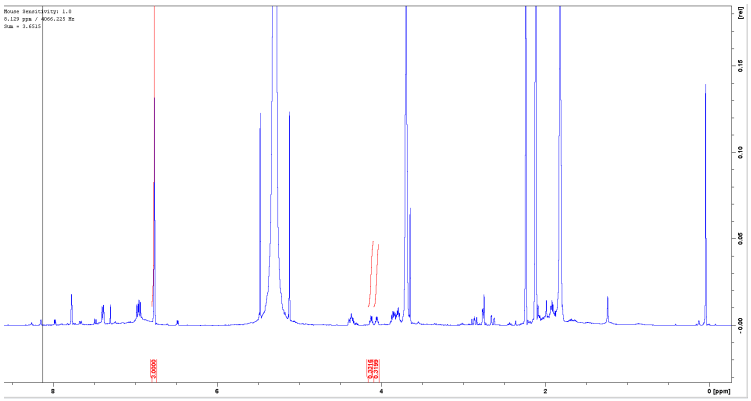
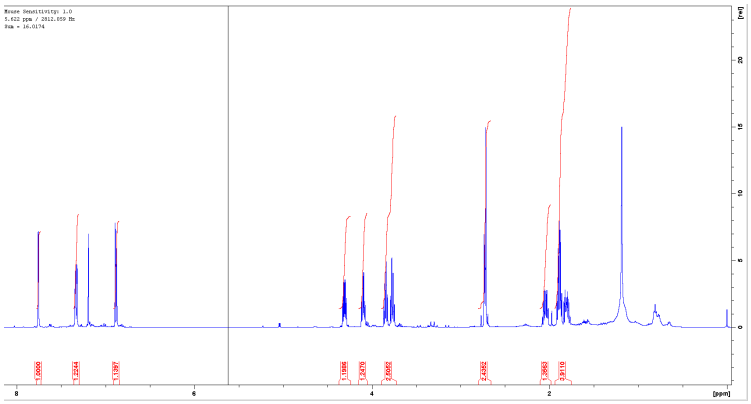


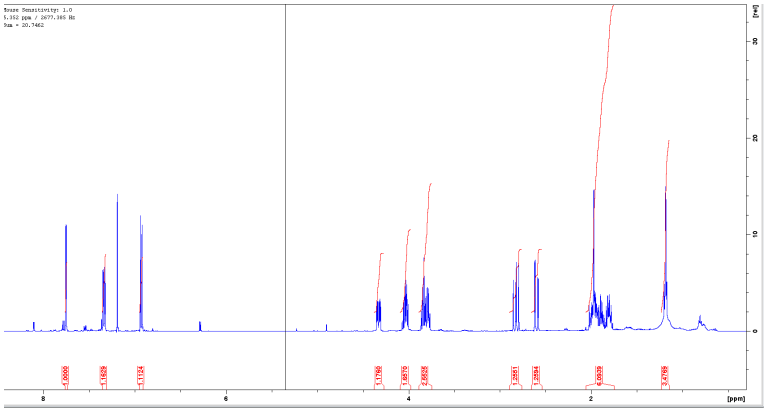
12 Crude



12 Top



12 Bottom



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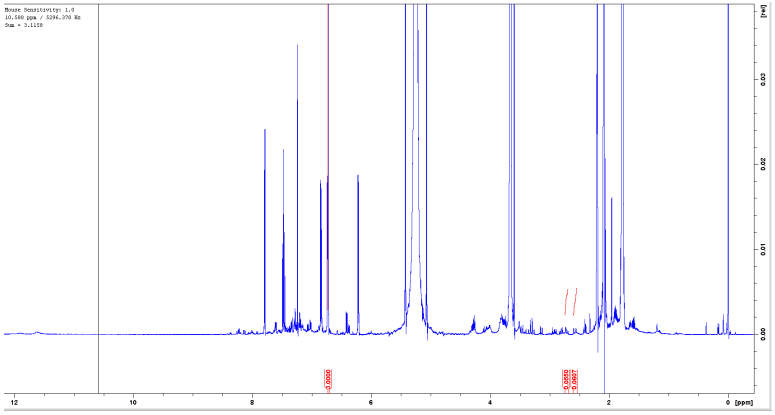
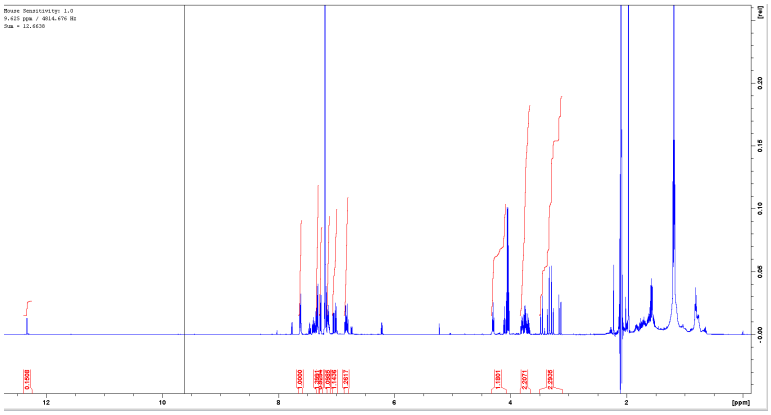
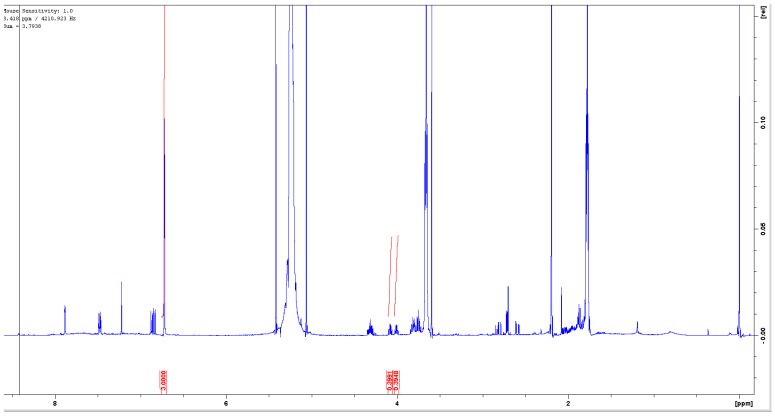


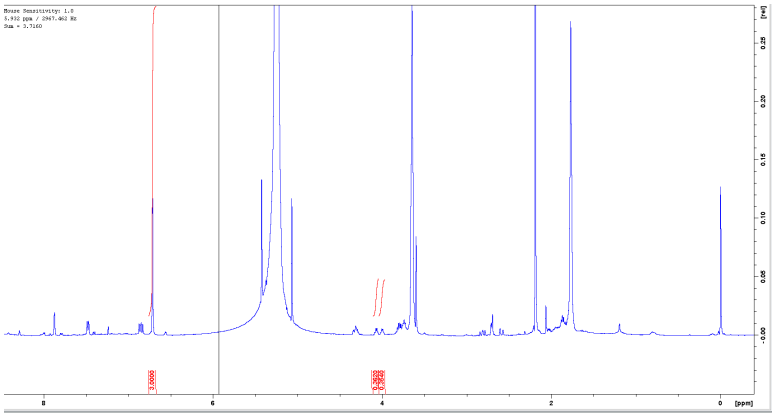
11 Spot



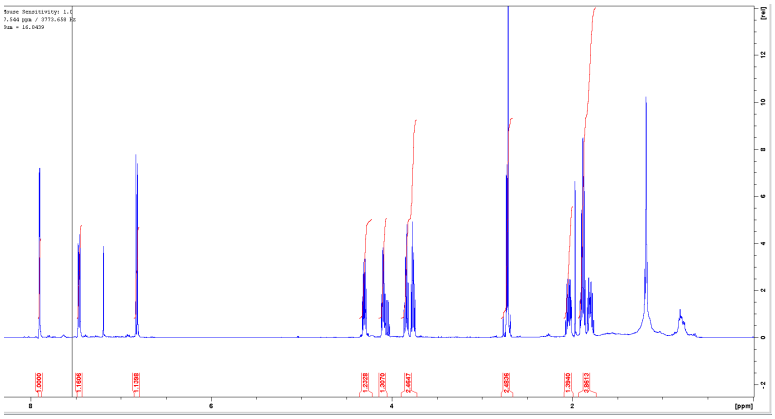
13a Crude



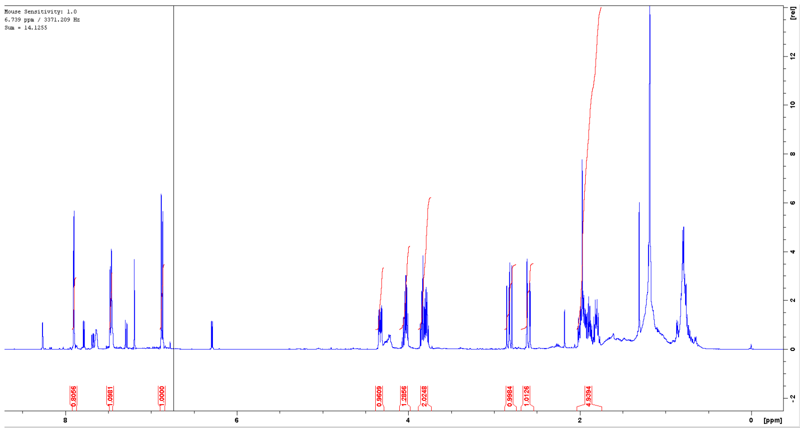
13b Crude



13 Top



13 Bottom



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1.0981
1.0000
0.9609
1.2856
2.0248
0.9984
1.0126
4.9394
8
6
4
2
0
[ppm]
[rel]
12
10
8
6
4
2
0
-2

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