Alkynones & Chalconoids as Bacterial Antagonists

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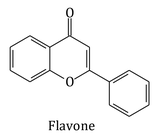
ABSTRACT

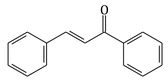
The general synthesis of conjugated alkynones has been reported by our research group previously, as well as their platinum-catalyzed regiospecific conversion to conjugated keto-vinyl ethers (chalconoids). We now report the synthesis and purification of two specific alkyones and their methoxy-chalconoid compounds. These compounds, along with commercially available flavone, were then applied in a serial dilution series of tests against two different bacterial strains [MRSA (Gram-positive) and Pseudomonas (Gram-negative)] to determine their cytotoxicity. This work demonstrates that further investigation is warranted for these classes of compounds, and their application as effective anti-bacterial, anti-oxidant, and potentially as anti-tumor agents.

BACKGROUND

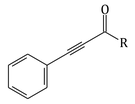
Flavone and flavonoid derivatives have shown promise as antimicrobial agents, particularly against antibiotic -resistant bacteria. They can disrupt bacterial membranes, inhibit efflux pumps, and even interfere with bacterial virulence factors.[1]



Flavone



Chalconoid

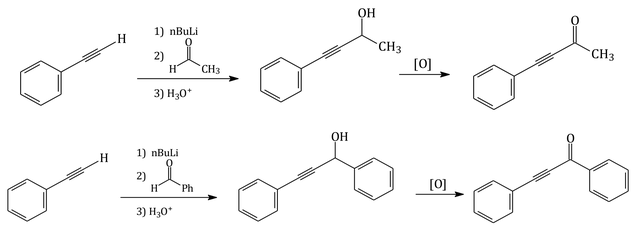


Conjugated Alkynone
(R = methyl or phenyl)

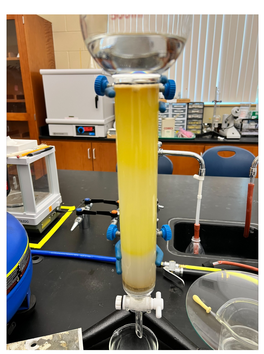
Structurally similar chalconoids are being explored as potential broad-spectrum antimicrobial agents, showing promise against bacteria, fungi, and even viruses. They are particularly attractive because they can disrupt bacterial cell membranes.[2] Our research group has successfully synthesized two conjugated alkynone compounds (precursors to the chalconoid structure) and employed a platinum(II)-catalyzed, regioselective addition to the alkynone to produce methoxy-substituted chalconoids.

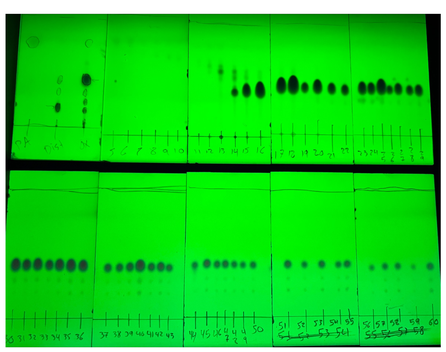
Alkynone and Chalconoid Synthesis

The two conjugated alkynones were synthesized in good yields as shown below:



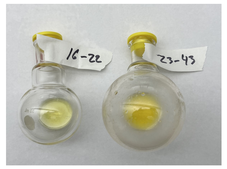
While the transformation of phenylacetylene into the alkynyl alcohols was routine, the subsequent oxidation to yield the alkynones proved to be more troublesome. Attempts to use bleach solutions in various solvents were low-yielding and left significant amounts of unreacted alkynyl alcohol substrate even with excess bleach. The Jones oxidation was then used with good yields and very little remaining starting material. IR and TLC analysis showed the presence of a few other impurities as well. Dichloromethane proved to be an ideal solvent system for the TLC analysis and subsequent column chromatography to purify the conjugated alkynone products.

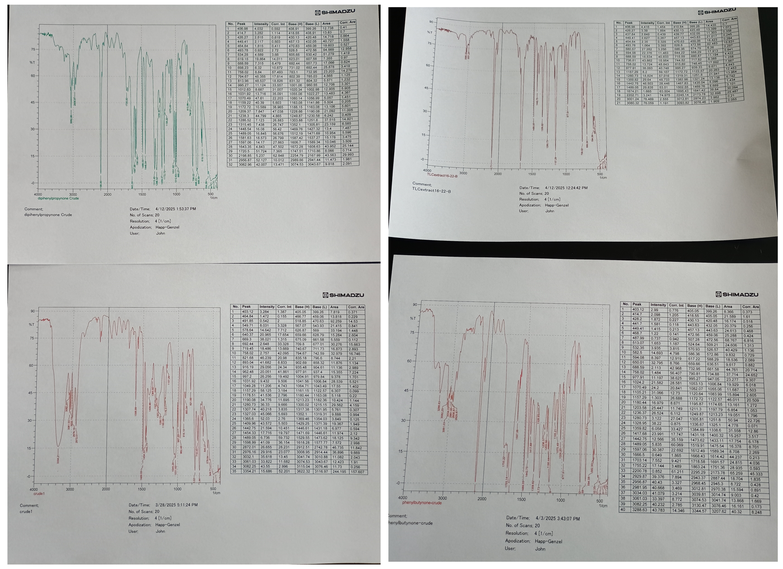




Isolation and Characterization

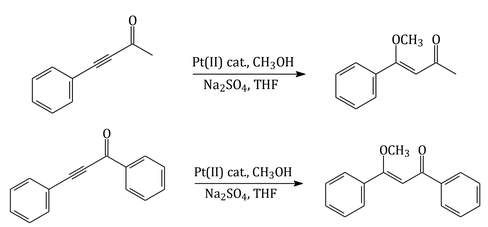
Sixty 3-mL samples were collected during the column chromatography of the crude alkynone after the oxidation reaction step. TLC analysis of these 60 samples showed that samples 16 through 22 consisted of pure alkynone, free of any starting material or other impurities. These seven samples were then combined, solvent evaporated, to yield the pure alkynone which was then analyzed by IR spectroscopy.





Platinum(II)-catalyzed addition of methanol

Each alkynone was then submitted to platinum(II)-catalyzed (in the form of THF-soluble Zeise's Dimer) addition of methanol [3,4] to yield the methoxy-substituted chalconoids:

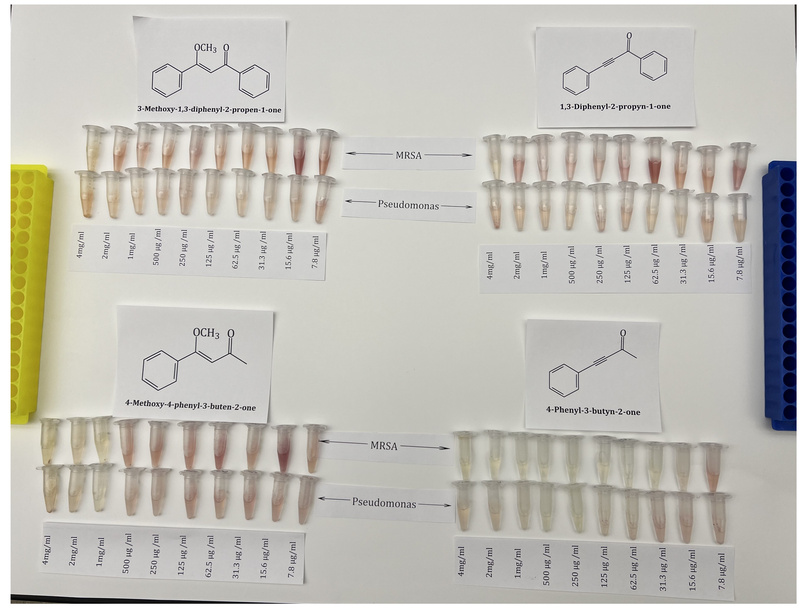


After removal of the platinum catalyst and solvent, the chalconoid products were isolated and analyzed via IR spectroscopy to confirm that the alcohol had added to the alkyne (absence of the carbon-carbon triple bond).

Antibacterial Activity

In collaboration with HC Molecular Biologist Phillip Sawatzki, broth media and two different strains of bacteria were prepared according to McFarland Standard protocol to ensure equivalent colony concentrations in each test vial. Unfortunately, since the broth media is aqueous based and flavone and our synthesized compounds are relatively non-polar organic compounds, solubility was a significant obstacle in these tests. For example, flavone is soluble in DMSO (dimethyl sulfoxide) solvent, but as soon as it is added to the broth media (itself containing about 5% DMSO), the flavone would precipitate out of solution and proved ineffective against either of the bacterial strains.

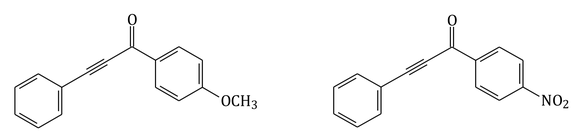
However, all four of our synthesized compounds demonstrated better solubility in the 5% DMSO/broth mixture than flavone and serial dilution assays were performed to determine the minimum inhibitory concentration (MIC) for each of the compounds. Each dilution series began at 4 mg compound per 1 mL solution down to 7.8 μg per 1 mL solution.



After inoculation of the bacterial solution with the appropriate dosage of the compound, the samples were incubated for 24 hours. Then after the incubation period, each vial was treated with a dye that indicates the presence of live bacteria. Red or pink color indicates live bacteria are present, while colorless solution indicates the bacteria have been killed/inhibited. The results indicate that the most effective compound was the alkynone 4-phenyl-3-butyn-2-one (with an MIC of 31 μg/mL against MRSA and 250 μg/mL against pseudomonas, while its corresponding chalconoid displayed attenuated activity with an MIC of 1 mg/mL against both bacterial strains. The diphenyl alkynone compound exhibited very poor antibacterial activity (4 mg/mL) against MRSA and no effectiveness against pseudomonas.

Conclusion

The results indicate that conjugated alkynones do exhibit antibacterial activity and warrant further investigation. Since the methoxy-substituted chalconoid showed less activity than its alkynone parent seems to indicate that the polarity of the core functional group (the ynone versus the keto enol ether) is inherent to the antibacterial activity. Likewise, the lack of activity displayed by the diphenyl alkynone may be explained by the difference in the electronics of that core functional group. This aspect can be investigated in future work of this project to expand the library of these synthesized conjugated alkynone substrates to include electron-donating and electron-withdrawing substituents on the aromatic rings like those shown below.



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Acknowledgements

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