Biorational pesticides: Pest control inspired by natural compounds

James Klimavicz
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

Recommended Citation
Klimavicz, James, "Biorational pesticides: Pest control inspired by natural compounds" (2020). Graduate Theses and Dissertations. 18159.
https://lib.dr.iastate.edu/etd/18159

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Biorational pesticides: Pest control inspired by natural compounds

by

James Scott Klimavicz

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-majors: Toxicology; Organic Chemistry

Program of Study Committee:
Joel R. Coats, Co-major Professor
George K. Kraus, Co-major Professor
Steven P. Bradbury
Philip M. Dixon
Richard J. Martin
Arthur H. Winter
Yan Zhao

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2020

Copyright © James Scott Klimavicz, 2020. All rights reserved.
DEDICATION

To my grandmother, Gloria Klimavicz, with love.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOMENCLATURE</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER 1. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Overview</td>
<td>1</td>
</tr>
<tr>
<td>Arthropod Repellents</td>
<td>3</td>
</tr>
<tr>
<td>Nematicides</td>
<td>7</td>
</tr>
<tr>
<td>The Role of Chemistry in Developing Biorational Compounds</td>
<td>10</td>
</tr>
<tr>
<td>Dissertation Outline</td>
<td>12</td>
</tr>
<tr>
<td>References</td>
<td>14</td>
</tr>
<tr>
<td>CHAPTER 2. MONOTERPENOID ISOVALERATE ESTERS AS LONG-LASTING SPATIAL MOSQUITO REPELLENTS</td>
<td>21</td>
</tr>
<tr>
<td>Abstract</td>
<td>21</td>
</tr>
<tr>
<td>Introduction</td>
<td>22</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>25</td>
</tr>
<tr>
<td>Chemicals and Synthesis</td>
<td>25</td>
</tr>
<tr>
<td>Repellency Assay</td>
<td>26</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>28</td>
</tr>
<tr>
<td>Isovalerate Repellency</td>
<td>28</td>
</tr>
<tr>
<td>Diffusion Modelling</td>
<td>32</td>
</tr>
<tr>
<td>Future Work</td>
<td>35</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>35</td>
</tr>
<tr>
<td>References</td>
<td>35</td>
</tr>
<tr>
<td>Appendix</td>
<td>38</td>
</tr>
<tr>
<td>CHAPTER 3. ESTERS OF CITRONELLOL AND CITRONELLIC ACID AS MOSQUITO REPELLENTS</td>
<td>42</td>
</tr>
<tr>
<td>Abstract</td>
<td>42</td>
</tr>
<tr>
<td>Introduction</td>
<td>43</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>45</td>
</tr>
<tr>
<td>General information</td>
<td>45</td>
</tr>
<tr>
<td>Citronellyl and citronellate ester synthesis</td>
<td>45</td>
</tr>
<tr>
<td>Mosquito repellency testing</td>
<td>54</td>
</tr>
<tr>
<td>Data analysis</td>
<td>55</td>
</tr>
<tr>
<td>Results</td>
<td>55</td>
</tr>
<tr>
<td>Discussion</td>
<td>61</td>
</tr>
<tr>
<td>Conclusion</td>
<td>64</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>64</td>
</tr>
</tbody>
</table>
## NOMENCLATURE

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABZ</td>
<td>Albendazole</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>ATTD</td>
<td>5-Alkylthio-1,2,4-thiadiazole</td>
</tr>
<tr>
<td>BC</td>
<td>Brain-Cousens</td>
</tr>
<tr>
<td>CRS</td>
<td>Cedargreen-Ritz-Streibig</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-Diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>DBMP</td>
<td>Dialkyl bis(alkylthio)methylphosphonate</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N’-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DCU</td>
<td>N,N’-Dicyclohexylurea</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>DEET</td>
<td>N,N-Diethyl-m-toluamide</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMPU</td>
<td>N,N’-Dimethylpropyleneurea</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>FDA</td>
<td>Fluorescein diacetate</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-Aminobutyric Acid</td>
</tr>
<tr>
<td>HWE</td>
<td>Horner-Wadsworth-Emmons</td>
</tr>
<tr>
<td>IR3535</td>
<td>Ethyl N-acetyl-N-butyl-β-alaninate</td>
</tr>
<tr>
<td>KDTA</td>
<td>Ketene dithioacetal</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>HRMS</td>
<td>High-resolution mass spectrometry</td>
</tr>
<tr>
<td>MTBE</td>
<td>Methyl tert-butyl ether</td>
</tr>
<tr>
<td>MTT</td>
<td>2-((4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>NCS</td>
<td>N-Chlorosuccinimide</td>
</tr>
<tr>
<td>NGM</td>
<td>Nematode growth media</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NTX</td>
<td>Nitazoxanide</td>
</tr>
<tr>
<td>PMD</td>
<td>p-Menthan-3,8-diol</td>
</tr>
<tr>
<td>PY</td>
<td>Pyrantel</td>
</tr>
<tr>
<td>QTOF</td>
<td>Quadrupole time-of-flight</td>
</tr>
<tr>
<td>RKN</td>
<td>Root-knot nematode (<em>Meloidogyne incognita</em>)</td>
</tr>
<tr>
<td>SCN</td>
<td>Soybean cyst nematode (<em>Heterodera glycines</em>)</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

The research in this dissertation would not have been possible without the contributions of many individuals. I would like to thank my co-major professors, Drs. Joel Coats and George Kraus, and my committee members, Drs. Steven Bradbury, Philip Dixon, Richard Martin, Arthur Winter, and Yan Zhao, for their guidance throughout this research, and for their support through the complexities of graduate school, ranging from lab work to paperwork. Additionally, I would like to thank Drs. Sarah Cady and Shu Xu for their work in maintaining the Chemical Instrumentation Facility, and for their training and valuable advice for acquiring NMRs.

The past and present members of the Coats Lab have been vital in driving the research group forward, and I appreciate all of their efforts, which helped to lay the groundwork for the research in this dissertation. In particular, Maura J. Hall’s knowledge, insight, and expertise led to fruitful conversations and helped to keep the lab running while also keeping me sane during my PhD program. Caleb L. Corona was an essential contributor to the progress made on mosquito repellents through his testing of synthesized compounds, and his work is greatly appreciated. Claire M. Pouliot worked countless hours in the Coats lab under my supervision, and her work towards maintaining the *C. elegans* colonies and performing related bioassay work was essential for the nematicidal studies performed during my program.

Much of this research was the result of collaboration. A collaboration with the Gregory L. Tylka Lab at Iowa State led to the discovery of new nematicidal compounds and the development of new methods for synthesis of sulfur-containing compounds. Jefferson O. Barizon’s work with soybean cyst nematode in the Tylka lab was essential to this project, and I am very thankful for his endless hours counting nematode eggs through a microscope. Dr. Philip
Dixon provided many valuable conversations about statistics and how best to approach much of my data analysis.

I would also like to thank my parents, Paul and Nancy Klimavicz, for their unwavering love and support throughout my life. I would not have been able to complete my graduate studies without their guidance, encouragement, and financial assistance. My grandparents, Gloria Klimavicz and Joyce and Robert Heins, and the rest of my family were also incredibly supportive. Lastly, I would like to thank all of my friends, my colleagues, the Entomology, Toxicology, and Chemistry faculty and staff, and the Graduate College for making my time at Iowa State University a fantastic experience.
ABSTRACT

Over the last several hundred million years, plants have evolved to produce an incredible variety of secondary metabolites. Many of these compounds are repellent or insecticidal and are generated by the plant to reduce herbivory. However, these compounds frequently lack the ideal chemical and physical properties that would make them suitable for larger-scale use as insect repellents or agricultural insecticides. Using a biorational approach, it is possible to chemically modify these natural metabolites to produce new compounds that are more ideal for commercialization.

Plant essential oils often contain a variety of monoterpenoids in high abundance, and these components of the oil are usually very fragrant. Although some of these compounds may be highly repellent or toxic to insects, the high volatility of these chemicals typically makes them unsuitable for use as long-lasting spatial insect repellents. The first part of this dissertation explores the chemical modification of monoterpenoid alcohols by esterification, which leads to compounds with higher molecular weight, and a concomitant drop in volatility, while maintaining satisfactory repellency characteristics against Culex pipiens, the northern house mosquito. Slight changes in the structure of the parent monoterpenoid or the esterifying group can often lead to substantial differences in potency of the repellency, and several of the synthesized compounds were repellent for more than seven hours against C. pipiens.

Plant-parasitic nematodes are a tremendous economic burden throughout the world, and although several terpenoids and phenylpropanoids have been shown to be somewhat toxic to some of these nematodes, treatment of infected soil is still often cost-prohibitive. A biorational approach was used to develop analogs of cinnamaldehyde, a compound previously shown to be effective against multiple species of nematodes in in vitro assays. Many of these compounds
were significantly more potent against the soybean cyst nematode (*Heterodera glycines*) than the parent compound.

The synthesis of analogs of a natural compound is an integral part of the biorational design of pesticides. The last portion of this dissertation focuses on two new synthetic methods, one for producing ketene dithioacetals, and one on the synthesis of 1,2,4-thiadiazoles. Both of these classes of compounds have been underexplored in agricultural chemistry, and the existing methods of creating these chemicals are limited. The new methods make these compounds in acceptable-to-good yields from readily-available starting materials.
CHAPTER 1. GENERAL INTRODUCTION

Overview

For as long as plants and herbivores have coexisted, an evolutionary arms race has been taking place, with plants developing defenses to reduce herbivory, and herbivores overcoming these adaptations. Direct plant defenses include physical adaptations, such as the development of spines, hairs, or waxy cuticles, as well as the production of plant secondary metabolites, which are compounds produced by plants that are not essential for the growth of the plant but improve the odds of plant survival by decreasing palatability of the plant. Indirect defenses do not directly impact the herbivore, but may instead attract predators or parasitoids through the release of volatile secondary metabolites; these natural enemies then attack the herbivore.\textsuperscript{1-2}

The secondary plant metabolites used in host defense are, chemically speaking, astonishingly diverse, representing many broad classes of compounds, including plant phenolics, flavonoids, terpenoids, phenylpropanoids, alkaloids, cyanogenic glycosides, glucosinolates, and polyketides. Indeed, the strongly pesticidal and repellent nature of many of these compounds have long been known. The extracts of \textit{Nicotiana spp.}, brought from the New World and containing the alkaloids nicotine and anabasine, had been used as insecticidal plant spray in Europe at least as early 1690, and nicotine sulfate was marketed as an insecticide beginning in 1910.\textsuperscript{3} While the use of nicotine (shown in Figure 1.1) as an insecticide has sharply declined due to its toxicity to mammals, the neonicotinoid class of insecticides was developed by mimicking the mode of action of nicotine.

Rotenone (shown in Figure 1.1) and the rotenoids are isoflavones derived from plants in several genera in family Fabaceae and possess extremely potent insecticidal and piscicidal
properties. The powdered roots of plants from *Derris sp.* have been used as an insecticide for centuries; until recently, rotenone was permitted for use on organic crops as a natural pesticide.

Pyrethrum, the dried flowers of *Chrysanthemum cinerariifolium* and *C. coccineum* (Asteraceae), has been used as an insecticide since antiquity, and was introduced into Europe in 1828. The extract of pyrethrum contains pyrethrins (Figure 1.1), a group of structurally similar natural terpenoid derivatives that are highly toxic to insects while showing minimal toxicity to mammals, birds, and plants. Similar to the way that nicotine inspired the development of neonicotinoids, natural pyrethrins led to the later development of the pyrethroid class of insecticides.

Despite the existence of some important classes of insecticides that were discovered without having a natural analog, such as DDT and the organochlorine insecticides, many new classes of insecticides are developed based on natural products. While a compound being described as “natural” by no means implies that this compound is safe to humans, plant secondary metabolites and other natural compounds will likely continue to be an essential source of inspiration for the development of pesticides with new modes of action.

---

*One need not look hard for a counterexample to this common misconception; nicotine is a fantastic insecticide, but is approximately 5,000-fold more toxic to mammals than to flies on a by-weight basis.*

Figure 1.1. Some of the pesticides used in the 19th and early 20th centuries. Natural pyrethrin is a mixture of six different compounds with \( R = \text{CH}_3 \) or \( \text{CO}_2\text{CH}_3 \) and \( R' = \text{CH}_3, \text{CH} = \text{CH}_2, \) or \( \text{CH}_2\text{CH}_3. \)
The Coats Lab at Iowa State has long been interested in some of the volatile compounds produced by plants as secondary metabolites—over the past several decades, research in the group has been focused on the insecticidal and repellent properties of volatile mono- and sesquiterpenoids, including nepetalactone and elemol, as well as some derivatives of these compounds. Continuing in this scope, the group has continued to work with biorational compounds inspired by naturally-occurring terpenoids and phenylpropanoids.

**Arthropod Repellents**

The use of plant essential oils dates back to antiquity, with references in ancient Greek, Roman, and Chinese documents. While it has long been known that these oils have culinary, therapeutic, and pesticidal applications, the first systematic investigations into the compositions of essential oils did not begin until the early- to mid-19th century, with Jean-Baptiste Dumas publishing one of the earliest papers on the separation and analysis of hydrocarbons and oxygenated compounds in a variety of oils and extracts, including turpentine, peppermint oil, and citron oil. The repellent nature of these oils to insects has long been known; citronella oil was reportedly used as a mosquito repellent as early as 1882.

Vincent Dethier, an expert in the field of plant-insect interactions, was one of the first scientists to systematically research insect attractants and repellents, particularly those found in plants. According to Dethier, and summarized again more recently by Katz *et al.*, there are multiple theoretical criteria that must be met for an ideal repellent. These requirements include the

![Chemical structures](image_url)

Figure 1.2. Mosquito repellents commonly used before the development of DEET. A common formulation used 3 parts, dimethyl phthalate, 1 part indalone, and 1 part 2-ethyl-1,3-hexanediol (called Rutgers 612 at the time).
protection of the treated surface for several hours under varying environmental conditions from all biting insects, a lack of toxicity or irritation to treated skin with no detrimental effect to clothing or other inert surface, no unpleasant odor or oily residue, easy application, and low cost. Of these, Dethier notes that the lack of an adverse effect upon application is by far the most important criterion, as a repellent is unlikely to be used if it irritates the skin or damages inert materials, such as plastics or synthetic fibers. Indeed, these problems were partially responsible for the poor compliance with repellent applications during World War II, particularly in the tropical theaters of the war, as the available repellents, shown in Figure 1.2, were irritating to the skin and destructive to plastics.\textsuperscript{18-21}

The development of \textit{N,N}-diethyl-\textit{m}-toluamide (DEET) near the end of World War II was the first in a new era of insect repellents. In the 11 years after the war, more than 20,000 compounds were tested for mosquito repellency in a massive screening effort to develop new, more tolerable repellents; of these, only a small fraction proved successful at repelling insects, and even fewer were tolerable to skin and surfaces. Some of the more promising early compounds included \textit{N}-butylacetanilide and \textit{N}-propylacetanilide.\textsuperscript{21} The DEET-like molecule \textit{N,N}-diethylbenzamidine was patented for use as an insect repellent in 1944,\textsuperscript{22} with DEET itself
being disclosed in a later publication in 1954.\textsuperscript{23} DEET was finally marketed to the public beginning in 1956, and the program to screen thousands of compounds for their ability to repel mosquitoes slowed significantly.

There are a variety of insect and arthropod repellents currently on the market. For the prevention of mosquito bites, particularly within the context of preventing the spread of Zika virus,\textsuperscript{†} the CDC recommends the use of DEET, ethyl N-acetyl-N-butyl-β-alaninate (IR3535), picaridin, p-menthane-3,8-diol (PMD), oil of lemon eucalyptus (which contains PMD), or 2-undecanone as repellents for skin use, or permethrin as an insecticide for use on clothing;\textsuperscript{24-25} the structures of these compounds are shown in Figure 1.3.

IR3535 was patented in 1974,\textsuperscript{26} and much like DEET, contains an amide functional group. Picaridin, patented in 1988,\textsuperscript{27} contains a carbamate functional group but behaves similarly to IR3535 and DEET. PMD is a dihydroxylated monoterpenoid, while 2-undecanone is an aliphatic ketone found as a primary constituent in rue oils.\textsuperscript{28} Citronella candles are currently on the market, and have a reputation for acting as efficient mosquito repellents; however, there is little concrete evidence to suggest that burning these candles is particularly effective at preventing insect bites.

Dethier, in a 1960 review,\textsuperscript{29} defines a repellent to be a compound that causes a target organism to actively avoid the source of the compound through an “action-at-a-distance”, implying that some quantity of the compound has evaporated, producing a region with vapor that is undesirable for the insect to enter. There is some anecdotal evidence to suggest that compounds with normal boiling points in the range of 230 to 260 °C make for ideal repellent candidates, as they appear to have high enough volatility to have potential spatial repellency,

\textsuperscript{†} Zika virus is a \textit{Flavivirus} spread by \textit{Aedes spp.}
though low enough to not evaporate too quickly.\textsuperscript{20,30} Many of the CDC-recommended repellents are estimated to boil near the upper end of this range,\textsuperscript{31} and these compounds are therefore likely to have lower vapor pressures than the “ideal” repellent; however, given that these compounds are typically viewed as contact irritants, this low vapor pressure may be beneficial. 2-Undecanone boils at 232 °C, and is more likely to act as a spatial repellent. However, this compound has a strong odor and is also used as a cat and dog repellent;\textsuperscript{31} it is left to the reader to determine if this is an advantage or disadvantage of use. Interestingly, under Dethier's definition, many of the repellents mentioned thus far, including DEET, picaridin, IR3535, and PMD, are not repellents \textit{sensu stricto}, as they are not volatile enough to vaporize to an appreciable extent; however, these compounds are still effective at preventing bites through their action as contact repellents.

There has been a recent push to remedy this apparent paradox through the use of new terms. Grieco differentiated “contact irritants” and “spatial repellents”, in which the former term refers to compounds that, like DEET, reduce the amount of time that mosquitoes and other arthropods remain on a treated surface; the latter term is in line with Dethier's definition of a repellent.\textsuperscript{32} Other terminological schemes include that used by Miller in an update to Dethier's initial semantics, with the terms engagent and disengagent for compounds that promote or diminish proximity of a responder to a stimulus, respectively.\textsuperscript{33} This distinction is in the spirit of Dethier's original work in which repellents are assumed to act in physiologically similar mechanisms, even when the behavioral mechanism is not known. As the focus of this project has been on the chemical synthesis of new repellents and not on the mechanisms through which
these compounds affect mosquito behavior, the terms “contact irritant” and “spatial repellent” will be used loosely herein.‡

A significant hurdle to the development of effective spatial repellents lies in the fact that they disperse readily by their nature, especially when there is significant air movement or if the treated surface is warm, as they must vaporize quickly enough to produce a repellent vapor. Evaporation of the compound leads to rapid dilution of the repellent as the vapor mixes with a large quantity of air. Citronella candles suffer from these problems—while the smoke and vapor from the candle contain a variety of repellent compounds, substantial convection from the hot candle flame causes rapid dispersal of the repellent compounds, an effect exacerbated by any wind present. However, it is worth noting that under carefully controlled static air conditions, citronella oil and many other plant essential oils are highly effective as insect repellents. While these oils tend to be highly complex mixtures of monoterpenoids, sesquiterpenoids, phenylpropanoids, and various other compounds, in many cases, much of the repellent activity can be attributed to a handful of these compounds. Identification of these spatial repellents and contact irritants allows us to use these compounds as starting points in developing improved disengagents. Thus far, the Coats lab has found that the majority of these promising compounds are terpenoid and phenylpropanoid alcohols.

**Nematicides**

Each year, plant-parasitic nematodes cause massive economic loss by reducing crop yield. Of particular interest in Iowa is the soybean cyst nematode (*Heterodera glycines*, SCN), an

---

‡ Permethrin and other pyrethroids are not really repellents under any of these definitions, since these compounds tend to affect the nervous system of the insect, and induce knockdown, wherein the insect is incapable of controlled flight or movement. The insect is therefore unable to behave in a manner indicative of repellency, such as steering away from the source or disengaging from the stimulus. However, for the intents and purposes of the person using the repellent, permethrin is a perfectly adequate “repellent” as it prevents mosquito bites. Many pyrethroids also display contact irritancy properties,34-35 which also deter biting but do not fall under a strict definition of repellency.
invasive species introduced into the southeast United States from Asia that has since become widespread in soybean-producing areas of the eastern US.\textsuperscript{36} \textit{H. glycines} can cause soybean crop losses in excess of 30\%; over the five-year period of 2010 – 2014, SCN resulted in the loss of approximately 617 million bushels of soybeans, equivalent to around $7.5 billion in damages.\textsuperscript{37}

Other nematodes, such as root-knot (\textit{Meloidogyne spp.}), root lesion (\textit{Pratylenchus spp.}), and foliar (\textit{Aphelenchoides spp.}) nematodes, are also economically relevant, both in commodity crops, such as soybeans and cotton, as well as higher-valued crops, such as cucurbits, citrus crops, stone fruits, peppers, tomatoes, potatoes, and many other fruits and vegetables.\textsuperscript{38}

Historically, commodity crops have not often been treated for nematode diseases, as the relatively low market value for these crops results in a high economic threshold for treating the field with nematicides, and it is therefore usually not economically beneficial to the farmer to use nematicides. In many fruit and vegetable crops, nematicides have been far more frequently used, as the increased yields of these high-valued crops merit the added cost of applying the pesticide.

Many traditional pesticides used to kill nematodes are actually soil sterilants, and include highly toxic alkylating agents. Methyl bromide was widely used across the US as a cheap and effective soil fumigant, killing nematodes and other pathogens alike. The use of methyl bromide in most agricultural settings was banned under the Montreal Protocol in 2004, though the US government still permits several critical use exemptions, including for Californian strawberry growers and producers of Virginia ham, as well as for quarantine and pre-shipment purposes. 1,3-Dichloropropene, chloropicrin, and metam sodium and dazomet (which produce methyl isothiocyanate on decomposition in soil) are still permitted for use as fumigants, but are less attractive due to higher costs and lower efficacy than methyl bromide.\textsuperscript{39} There are several other pesticides that have been labelled for use against nematodes. Aldicarb is a carbamate pesticide
Effective against many insects and nematodes but is extremely toxic to mammals.\textsuperscript{\S} Other alternatives include the natural product abamectin, the organophosphates fosthiazate (Syngenta: Nemathorin; predominantly Europe and Asia for crop use) and fenamiphos (no longer in use\textsuperscript{40}), fluopyram (Bayer: Indemnify; for turf use only), fluensulfone (Quali-Pro: Nimitz Pro G; for turf use only), and tioxazafen (Monsanto: NemaStrike; for crop and seed treatments). Tioxazafen is of particular interest because it is registered for use as a soybean seed coating for use against soybean cyst nematode.\textsuperscript{41} There are also a few other seed coatings meant to reduce the nematode burden on plants, and include the use of harpin proteins to induce plant natural defenses (Plant Health Care, Inc.: N-Hibit) and the use of \textit{Bacillus spp.} (Bayer: Votivo and Valent: Aveo) or \textit{Pasteuria nishizawai} (Syngenta: Clariva) as nematode repellents or nematicidal parasitic bacteria.\textsuperscript{42}

While there are several compounds currently registered for use against plant-pathogenic nematodes, the loss of methyl bromide and other effective nematicides, coupled with the

\textsuperscript{\S} Bayer's product Temik 15 contained 15% aldicarb, but the registration for this product was voluntarily withdrawn. AgLogic 15G, produced by AgLogic Chemical, is a similar product with strictly limited uses.
continuing economic losses from these pests, creates a serious need for new technology to combat nematodes. Using a biorational approach, we sought to develop new compounds as potential nematicides.

**The Role of Chemistry in Developing Biorational Compounds**

The definition of the term “biorational” remains somewhat fluid and field-dependent. In some cases, the emphasis is on compounds, oils, or formulations that are naturally-occurring with little impact on non-target organisms and the environment,\(^{43}\) while a more common, looser definition with a focus on finding naturally-occurring compounds that exhibit a specific mode of action, and then developing new pesticides, repellents, and pharmaceuticals that improve the specificity, efficacy, or potency of the parent scaffold.\(^ {44-47}\) This dissertation has embraced the latter, broader sense of the term; however, both strategies share the common goal of identifying natural compounds that exhibit desired activity against an organism or biomolecular target.

The entire process of developing biorational compounds is dependent on chemistry, from isolating a bioactive molecule and determining its structure and mode of action, to the development of new analogs. In some cases, the isolated compound is sufficiently active to be useful as a drug, such as the case with artemisinin, a highly oxygenated sesquiterpenoid first isolated from *Artemisia annua* (Asteraceae) that acts as a potent antimalarial drug.\(^ {48-49}\) Initially hampered by poor availability and affordability, the production of artemisinin has increased as a result of selective breeding and microbial fermentation pathways.\(^ {50}\) Additionally, artemisinin has resulted in the development of other antimalarial drugs, such as artemether, a semisynthetic analog with improved pharmacokinetic properties, produced directly from artemisinin via reduction and acetal formation. Artemisinin has also inspired a series of synthetic biorational antimalarials containing the unusual 1,2,4-trioxane structure.\(^ {51-54}\)
Artemisinin and its analogs are not isolated examples; indeed, the development of new analogs of natural compounds is an essential approach for the development of new pharmaceuticals. The importance of this method to the drug discovery process cannot be adequately conveyed in a short chapter, but only a few examples are needed to show the value of this strategy. The β-lactam antibiotics, which includes the semisynthetic biorational derivatives of fungal compounds like the penicillins, cephalosporins, carbapenems, and monobactams, are perhaps the most familiar drugs developed based on natural compounds and are still indispensable to proper function any health system. Likewise, the opioid drugs were inspired by alkaloids found in opium; this class of drugs includes some extremely potent painkillers. Tragically, many of these drugs are highly addictive, and the opioid epidemic in the United States has resulted in the death of nearly 400,000 people between 1999 and 2017. The opioid endemic has renewed the focus on the discovery of additional natural analgesics, and the biorational modification of these compounds to develop new therapeutic compounds with lower risks of addiction.

Many insecticides have been developed through a biorational approach. As discussed vide supra, the insecticidal activity of nicotine led to the neonicotinoid class of insecticides, which are far more potent against insects and less toxic to mammals. Likewise, the discovery of the insecticidal capacity of pyrethrins led to the development of pyrethroids. The compounds in both of these insecticide classes have far higher potency against insects than against mammals—neonicotinoids target the nervous system of insects with far greater specificity than that of mammals, while most mammals metabolize pyrethroids far faster than insect do, leading to the organismal selectivity. Methoprene and hydroprene are potent insect growth regulators developed through a biorational process as synthetic analogs to the acyclic sesquiterpenoid
juvenile hormones (JH);\textsuperscript{46} a particularly attractive starting scaffold as JH is insect-specific, and these analogs have low mammalian toxicity.\textsuperscript{70-72}

Spinosyns, the polyketide-derived macrocycles initially isolated from the actinomycete \textit{Saccharopolyspora spinosa}, led to the development of Spinosad, a mixture of the polyketides spinosyn A and D, as an effective insecticide against many lepidopteran pests.\textsuperscript{73-74} Strobilurins A and B, natural products first isolated from the fungus \textit{Strobilurus tenacellus},\textsuperscript{75} have inspired the strobilurin class of fungicides that are now commonly used in agriculture.\textsuperscript{76}

The development of novel biorational pesticides and antibiotics is reliant on chemical synthesis. New techniques and synthetic methods are often required to make the desired compounds, such as the synthesis of the first nitromethylene and chloronicotinyl insecticides,\textsuperscript{77-78} or the production of amoxicillin and other penicillin analogs from 6-aminopenicillanic acid.\textsuperscript{79-80} The synthetic pyrethroids likewise required new reactions to produce the desired analogs in acceptable yields.\textsuperscript{81} While the development of biorational pesticides and drugs relies on advances in organic synthetic methodology, the biological activity of secondary plant metabolites continues to serve as a source of inspiration for chemistry.

**Dissertation Outline**

As the Coats lab has long focused on both insect repellents and essential oils for pest control, the first portion of this dissertation explores derivatives of monoterpenoids as mosquito repellents. Although monoterpenoids on their own are rarely good repellents in long-term assays,\textsuperscript{**} many sesquiterpenoids, with their higher molecular weights and lower volatility, better serve as mosquito repellents. Chapters 2 and 3 of this dissertation explore monoterpenoid alcohols esterified with relatively small carboxylic acids to produce compounds comparable in

\textsuperscript{**} A notable exception to this is PMD, which is a monoterpenoid with low volatility—a result of its two free hydroxyl groups.
molecular weight and polarity to sesquiterpenoids. Chapter 4 investigates monoterpenoid carbonate esters as a variation on the carboxylate esters from Chapters 2 and 3.

Because many of the terpenoids and phenylpropanoids produced by plants are suspected to reduce herbivory from various organisms beyond class Insecta, an exploration of the nematicidal properties of these compounds was performed. In particular, many of these compounds were screened for their ability to inhibit egg-hatching rates of the economically important *H. glycines*. Because even an in vitro assay of egg-hatch inhibition in SCN is labor-and time-intensive, a higher-throughput assay with *C. elegans* eggs was developed to screen these compounds, using fluorescein diacetate as a metabolic indicator. The development of this fluorescence-based assay using *C. elegans* is discussed in Chapter 5, using a panel of monoterpenoids, phenylpropanoids, cinnamaldehyde analogs, and nematicidal compounds to assess the utility of the assay. Chapter 6 summarizes an investigation of the ability of biorational analogs of cinnamaldehyde to modulate that hatch rates of SCN, and compares the efficacy of these analogs in SCN and *C. elegans*. These analogs range from substituted cinnamaldehydes and β-nitrostyrenes, to styryl ketones and styryl sulfides, to phenylazoles, including pyrazoles, isoxazoles, and an isothiazole.

Chapters 7 and 8 discuss new synthetic methods that were developed in the course of developing cinnamaldehyde analogs. An attempt to make styryl sulfides led to the production of ketene dithioacetals, and an optimization of this reaction led to the results discussed in Chapter 7. In addition to potentially serving as nematicides, these compounds are potentially useful as synthetic intermediates, as ketene dithioacetals participate in a variety of reactions. The development of alkylthio-substituted phenylazoles as biorational analogs of cinnamaldehyde led to the discovery of a 5-(alkylthio)isothiazole with potent activity when screened against SCN and
C. elegans. The quest to make an analogous 5-alkythyio-1,2,4-thiadiazole ultimately led to a new procedure for synthesizing these compounds, which is discussed in detail in Chapter 8. The 1,2,4-thiadiazole ring is underexplored in the development of agricultural and pharmaceutical compounds, partly due to a lack of available reactions for their synthesis. The addition of this procedure to the chemical literature will improve the accessibility of these compounds for future examination.

References


7. Sparks, T. C.; Lorsbach, B. A., Agrochemical discovery - Building the next generation of insect control agents. In Advances in Agrochemicals: Ion Channels and G Protein-Coupled Receptors (GPCRs) as Targets for Pest Control, 2017; pp 1--17.


9. Rice, P. J.; Coats, J. R., Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 1994, 87, 1172-1179.


CHAPTER 2. MONOTERPENOID ISOVALERATE ESTERS AS LONG-LASTING SPATIAL MOSQUITO REPELLENTS

James S. Klimavicz,1* Caleb L. Corona,1 Edmund J. Norris,1 and Joel R. Coats1

1Department of Entomology, Iowa State University, Ames, IA, 50011

Modified from a peer-reviewed book chapter published in Biorational Control of Medical and Veterinary Pests, ACS Symposium Series, Vol. 1289, with permission, from Klimavicz et. al.1

Copyright 2018 American Chemical Society.

Abstract

Plant essential oils and their constituent monoterpenoids and phenylpropanoids have long been used as insect repellents. However, due to the low molecular weight of these compounds, vaporization is often rapid, leaving little residual repellent activity. Although sesquiterpenoids, as heavier, less volatile molecules, often have good repellent properties, these compounds are often less economically viable as repellents due to high production and isolation costs. With the objective of producing long-lasting repellents from cheaper starting materials, monoterpenoid and phenylpropanoid alcohols/phenols were esterified with isovaleric acid to produce a set of esters with molecular weights similar to sesquiterpenoids. A static-air repellency chamber was used to assay the repellency of the synthesized compounds against Culex pipiens. Most compounds showed significant repellency over the 150-minute duration of the short-term assay. Several compounds, particularly those derived from monocyclic monoterpenoids, retained substantial repellent effects in a long-term assay, indicating the potential for increased-duration

* JSK synthesized all compounds for testing, performed the modelling of diffusion of compounds in bioassay chambers, and wrote the manuscript. CLC performed most of the bioassay testing. EJN oversaw some of the initial bioassay testing. JRC assisted in the revision of the manuscript.
insect repellents. Trends in the short-term assay are partially explained using solutions to the diffusion equation.

**Introduction**

Plant essential oils, used since antiquity for their flavors and fragrances, have long been known to repel a variety of insect pests. The main constituents of the majority of these oils are a variety of terpenoids and phenylpropanoids. As secondary metabolites, these compounds serve a variety of purposes in plants; some, such as geraniol, have been identified as components in floral scents responsible for attracting pollinators while others, such as menthol and thymol, are potent insect antifeedants, repellents, or insecticides.²⁻⁵ Despite an abundance of synthetic repellents and insecticides used today, essential oils and terpenoids have found continued use as botanical alternatives due to the assumed environmental and human safety of these products.⁶ As examples, citronella oil, the essential oil from one of several *Cymbopogon* species, has been an EPA-registered insect repellent since 1948,⁷ while *p*-menthane-3,8-diol, a component of the lemon eucalyptus tree (*Corymbia citriodora*) essential oil, has been registered as a natural mosquito repellent with the EPA since 2000.⁸

As mosquitoes continue to cause significant worldwide morbidity and mortality, and insecticide resistance has become more widespread,⁹ there has been a renewed focus on determining the mode of action of insecticides currently in use.¹⁰ While monoterpenoids have long found use as insect repellents, the mechanism of action of repellency of most of these compounds has not been fully elucidated, and is currently a topic of research interest.⁵,¹¹⁻¹⁴ Further complicating the matter, compounds may exhibit spatial or contact repellency (or some combination thereof), suggesting multiple mechanisms by which these chemicals may act.¹⁵ The commonly-used *N*,*N*-diethyl-3-toluimide (DEET) acts much better as a contact repellent;¹⁶ both
DEET and \( p \)-menthane-3,8-diol are relatively poor spatial repellents, while citronella oil and other plant essential oils are often very good spatial repellents.\(^{17}\)

In plants, monoterpenoids and phenylpropanoids are secondary metabolites, often acting as allelochemicals. Monoterpenoids are \( C_{10} \) compounds derived from geranyl pyrophosphate,\(^{18}\) a product of the methyl erythritol pathway (MEP) or mevalonate pathway, while phenylpropanoid biosynthesis commences with the non-oxidative deamination of phenylalanine to form the nine-carbon cinnamic acid, which may then be oxidized to coumaric acid and other phenylpropanoids.\(^{19}\) Biosynthesis of monoterpenoids occurs through various enzyme-mediated carbocation rearrangements to form a wide variety of structural motifs;\(^{20}\) of interest in this study were the monoterpenoid alcohols produced upon quenching these carbocations with water. The carbocation rearrangements, as well as other modifications, produce the monoterpenoid and phenylpropanoid alcohols and phenols shown in Figure 2.1. The compounds of interest used in

![Figure 2.1. Structures of the monoterpenoid and phenylpropanoid alcohols and phenols used as starting materials for esters. Chiral compounds were used as racemic mixtures unless specified; carveol (1i) was purchased as a mixture of cis/trans isomers.](image-url)
this study include the acyclic monoterpenoids 1a-d, the monocyclic monoterpenoids 1e-k, the bicyclic monoterpenoids 1l-p, and the phenylpropanoids 1q-u, providing a variety of structural motifs for exploring the relationships between structure and repellent effects.

Due to their low molecular weights when compared to the C_{15} sesquiterpenoids, the monoterpenoids and phenylpropanoid alcohols tend to vaporize relatively quickly. As a result, many of these compounds show promising repellent effects over a short testing period, while they are in the process of evaporating from the treated surface. However, once the most volatile components have vaporized, the repellent effect of the compound or essential oil is typically greatly diminished. This effect is typified by citronella oil, shown in Figure 2.2. Citronella oil is mainly composed of citronellal, geraniol, citronellol, and several other monoterpenoids, resulting in a repellent with high short-term repellency but with little duration.

![Figure 2.2](image)

**Figure 2.2.** Comparisons of the short- and long-term repellency of citronella oil (Java origin). Error bars represent the standard error of the mean percentage repellency at each time point.
Esterification of repellent monoterpenoid and phenylpropanoid alcohols provides a way to increase molecular weight while maintaining the structure of the parent compound, which may result in repellents with a longer lifetime and much of the potency of the starting alcohol. In an effort to develop sesquiterpenoid-like esters capable of repelling mosquitoes over long periods, a series of 21 isovalerate esters was synthesized to explore the repellent properties of the parent monoterpenoids and phenylpropanoids upon esterification, allowing a biorational approach when targeting new esters for synthesis of repellents.

**Materials and Methods**

**Chemicals and Synthesis**

Monoterpenoid and phenylpropanoid alcohols and phenols were purchased from MilliporeSigma (formerly Sigma-Aldrich), unless otherwise specified, and were used without further purification. Citronellol, linalool, α-terpineol, isoborneol, and fenchol were purchased as racemic compounds, while (−)-menthol, (−)-isopulegol, (−)-perillyl alcohol, (−)-borneol, (−)-myrtenol, and (−)-verbenol (Acros) were purchased as single enantiomers. Geraniol and nerol were used as single stereoisomers. Isovaleric acid (MilliporeSigma), \(N,N'\)-dicyclohexylcarbodiimide (DCC, Oakwood Chemical), and 4-(dimethylamino)pyridine (DMAP, Oakwood Chemical) were also used without additional purification.

All esters were synthesized using a slight modification of the Steglich esterification (Scheme 2.1),\(^{21}\) which has been described previously.\(^{22}\) Typically, the alcohol or phenol (10

\[
\text{R OH} \quad \xrightarrow{\text{cat. DMAP}} \quad \text{R} \quad \xrightarrow{\text{cat. DMAP}} \quad \text{DCC} \quad \text{2a-u}
\]

Scheme 2.1. Synthesis of isovalerate esters 2a-u from monoterpenoids 1a-u and isovaleric acid via a Steglich esterification.
mmol), DMAP (1.5 mmol, 183 mg), and isovaleric acid or other carboxylic acid (12 mmol) were dissolved in dichloromethane (DCM, 15 mL) in a 125 mL Erlenmeyer flask open to air. The alcohol-containing solution was cooled to 0 °C, at which point a solution of DCC (10.5 mmol, 2.17 g) in DCM (10 mL) was added over 1 minute. The reaction was stirred at 0 °C for 15 minutes, and then at room temperature for 3 hours. Dicyclohexylurea (DCU) precipitated as a white solid; hexane (100 mL) was added, and the mixture was cooled to -20 °C overnight to precipitate the bulk of the DCU. The solid was removed by vacuum filtration and washed with hexane, and the filtrate was then reduced under vacuum to give the crude ester. The desired ester was purified by column chromatography, typically using 10:90 ethyl acetate/hexane; a more polar ratio of 15:85 was used for phenylpropanoid esters. Yields were typically in the range of 60 to 99%; yield of approximately 50% were noted for several tertiary alcohols. The identity and purity of all compounds were verified by NMR spectroscopy, and all compounds tested were at least 98% pure by NMR.

Synthesis of umbelliferone isovalerate required the use of a 1:1 mixture of DCM:dimethylformamide due to a lack of solubility in DCM. Workup of this reaction is performed by adding water (100 mL) and hexane (100 mL), and cooling to 0 °C. DCU was removed by filtration as before; the organic phase of the filtrate was isolated in a separatory funnel and washed with water (2x 100 mL) and then 1M sodium hydroxide to remove any remaining umbelliferone. The organic solution was washed with brine, dried over anhydrous magnesium sulfate, and reduced under pressure to give the ester as a crude solid, which could be recrystallized from 50:50 ethyl acetate/hexane.

Repellency Assay

The static air repellency chamber used in the repellency assay has been described previously.23-24 Briefly, the repellency chamber consists of a clear glass cylinder 600 mm in
length with an inner diameter of roughly 85 mm laying horizontally. One end of the cylinder is capped with an untreated 90-mm filter paper (Whatman No. 1) contained in a 90-mm inner diameter glass petri dish, and sealed with tape to remain closed. The other end is capped in the same manner, except the filter paper has been treated with 1 mL of a 0.5% (w/v) solution of a monoterpenoid ester in acetone, resulting in 78.6 μg cm$^{-2}$ of repellent on the filter paper. The solution was added to the filter paper evenly and at a rate such that the evaporation of acetone prevents loss of compound from dripping, and the acetone was allowed to evaporate at room temperature for a further 10 minutes before use for the short-term repellency assay, or for an additional five hours under open air in ambient conditions in the case of long-term repellency studies.

 Shortly after placing the filter papers on the end of the tube, twenty (not blood fed) female *Culex pipiens* mosquitoes were anesthetized with carbon dioxide, and placed into the tube via a 20-mm hole halfway down the length of the tube, and the hole was sealed. This process is performed with as little disturbance to the air in the tube as possible. The distribution of mosquitoes between the treated and untreated sides was then noted at 15, 30, 60, 90, 120, and 150 minutes after placement into the tube. Percentage repellency was determined with the formula

$$%_r = 100\% \cdot \frac{n_t - n_u}{n_t + n_u},$$

where $n_t$ and $n_u$ are the respective numbers of mosquitoes on the treated and untreated sides of the repellency chamber. In several assays, the chemical repellent caused some level of mosquito knockdown; the knocked-down mosquitoes were counted as if they were repelled on the premise that a knocked-down mosquito would be unable to feed on the host. This is also in line with evidence that highly repellent compounds often cause significant knockdown.$^{25}$
replicates were performed for each compound, and average repellency values were used. A test run was performed with acetone only to ensure that residue from the solvent did not have a significant repellent or attractant effect.

**Results and Discussion**

**Isovalerate Repellency**

**Short- and long-term repellency**

In the short-term assay, a range of repellent effects was observed, varying from nearly 100% repellency to an effect no different than the control, allowing differentiation between different compounds. Likewise, the long-term assay revealed particularly potent repellents with high repellency even after the treated surface was exposed to air for five hours; other, less-potent repellents performed only modestly after this time period. Classification of the isovalerate esters into acyclic, monocyclic, bicyclic, and phenylpropanoid esters allows for comparison of both the long- and short-term repellent effects of esters with similar structures.

The four acyclic monoterpenoid isovalerates, shown in Figure 2.3, all followed the same general trends, with increasing repellency over the duration of the short-term assay, giving moderately high percentage repellencies of 65-90%. The results in the long-term assay were more modest, with repellencies ranging from 30 to 75% after 60 minutes in the assay. Linalyl isovalerate (2d), a tertiary alcohol ester, performed numerically better in the short-term assay, which is potentially of interest given that the other acyclic esters are all derived from primary alcohols.

As shown in Figure 2.4, the monocyclic isovalerates also tended to perform well in the short-term and long-term assays. Menthol isovalerate (2e) was a conspicuous exception, with relatively poor repellency in both assays. The aromatic compounds thymyl isovalerate (2j) and carvacryl isovalerate (2k) were both substantially less repellent in the long-term assay than their
Figure 2.3. Short- and long-term repellency of the acyclic monoterpenoid repellents citronellyl isovalerate (2a), geranyl isovalerate (2b), neryl isovalerate (2c), and linalyl isovalerate (2d).

Figure 2.4. Short- and long-term repellency of the monocyclic monoterpenoid repellents menthyl isovalerate (2e), isopulegyl isovalerate (2f), α-terpinyl isovalerate (2g), perillyl isovalerate (2h), carvyl isovalerate (2i), thymyl isovalerate (2j), and carvacryl isovalerate (2k).
unsaturated alicyclic counterparts, isopulegyl isovalerate (2f) and carvyl isovalerate (2i); it is unclear if this difference is due to lower potency. Numerically, α-terpinyl isovalerate (2g), perillyl isovalerate (2h), and 2i all performed better after 60 minutes in the long-term assay than they did in the short-term assay; however, this is likely due to statistical variation or slight genetic differences between different cohorts of mosquitoes. Regardless, 2g-i all had very impressive long-term repellency, implying that these repellents are more potent than other monoterpenoid isovalerates. Additionally, 2g knocked down nearly 30% of the mosquitoes across the four long-term assays performed.

Overall, all bicyclic isovalerates tested showed substantial short-term repellency, though the long-term repellency of these compounds was moderate at best (Figure 2.5). The higher branching and more compact shape of these molecules may result in higher vapor pressures,26-27

Figure 2.5. Short- and long-term repellency of the bicyclic monoterpenoid repellents myrtenyl isovalerate (2l), verbanyl isovalerate (2m), bornyl isovalerate (2n), isobornyl isovalerate (2o), and fenchyl isovalerate (2p).
increasing repellency of these compounds in the initial phase of the short-term assay, but reducing the lifetime of the repellent, giving low-to-moderate long-term repellency. The use of a larger carboxylate residue may improve the lifetime of these compounds on a treated surface by decreasing volatility.

The phenylpropanoid repellents, as a group, were less impressive than the other isovalerate groups. Short-term repellencies were modest, and most of the long-term data showed only very slight, if any, repellency (Figure 2.6) showed some numerical attractancy, which is being further explored as this phenylpropanoid ester was the only coumarin derivative. Piperonyl isovalerate (2t) showed high repellency in the short-term assay, though performance in the long-
term assay was unremarkable. **2t** was of interest due to the structural relationship between this ester and piperonyl butoxide, a commonly used synergistic adjuvant for pyrethroids.

It is noteworthy that all three of the isovalerate esters showing greater than 90% repellency in the long-term assay (compounds **2g-i**) contain a monocyclic cyclohexene moiety; the bicyclic esters containing the cyclohexene structure did not show high levels of repellency in the long-term study. This structural relationship between these compounds may be indicative of a common mode of action or binding site of these repellents, and merits further study.

**Knockdown**

Several compounds resulted in the knockdown (partial paralysis or ataxia) of some of the mosquitoes across several trials, including **2g**, which induced knockdown in approximately 30% of the mosquitoes. Based on the continuous observation of several assays, the knocked-down mosquitoes appeared to experience ataxia most frequently after direct contact with the treated filter paper surface.

The implications of knockdown in this study are not entirely clear, as it is not currently known whether the compounds that cause knockdown are more insecticidal than those that do not. Pyrethroids and DDT cause knockdown in mosquitoes by binding to voltage-gated sodium channels. Given the development of knockdown-resistant mosquito strains that are resistant to DDT or pyrethroid insecticides through the development of point mutations,\(^{28-29}\) it is of interest to determine whether monoterpenoid esters will still induce knockdown in these resistant strains, which would implicate a different binding site.

**Diffusion Modelling**

The nonlinear repellency data with respect to time from the short-term static air chamber repellency assay is partially explained by modeling the diffusion of the repellent compound
Across the tube. As the glass chamber is sealed for the duration of the experiment, the concentration $\phi$ of the repellent can be roughly estimated using the diffusion equation

$$\frac{\partial \phi(r, t)}{\partial t} = D \nabla^2 \phi(r, t)$$

where $D$ is the diffusion coefficient, $t$ is time, and $r$ is the distance from the treated filter paper.

The setup of the repellency chamber permits the use of the 1-dimensional diffusion equation, as the treated filter paper is the diameter of the cross section of the glass tube. The diffusion coefficient is compound-dependent, and describes how quickly a compound will diffuse through the tube, with higher values implying faster rates of diffusion; a typical value for a monoterpenoid ester is on the order of $4.0–5.0$ mm$^2$ s$^{-1}$.30

Figure 2.7 shows the relative concentration profiles in the repellency chamber at the observational time marks used in the study. For the purpose of preliminary modeling of these compounds, a diffusion coefficient of $4.5$ mm$^2$ s$^{-1}$ was chosen, as this value is similar to the measured diffusion coefficients of similar molecular size. The relative concentration is the fractional concentration with respect to the concentration of repellent just above the surface of the treated filter paper. For the short-term assay, this concentration is assumed to be dependent on the vapor pressure of the compound and constant for the entire experiment. This second approximation appears to be inadequate for the long-term assay, however, as many repellents do not show the upward trend in repellency; it is likely that the bulk of the material has vaporized from the filter paper, and an insufficient quantity remains to saturate the air adjacent to the filter paper.

The curves in Figure 2.7 depict the repellent concentration gradient in the chamber at different time points, and the gradient is steepest near the treated surface. Until roughly 30 minutes has passed, there is negligible repellent vapor on the untreated half of the tube, though
this time point will vary with any turbulence introduced into the tube as well as the precise diffusion coefficient of the compound in question. The rapid change in concentration gradient at the start of the assay explains the typically sharp increase in repellency in the first 30 minutes. At approximately 90 minutes, the concentration of repellent at the untreated end of the tube starts to rise significantly, which helps to explain the leveling out of the percentage repellency beyond this point. This loss of concentration gradient across the length of the tube may also explain the slight numerical decrease in repellency seen in several compounds.

Figure 2.7. Solutions to the diffusion equation with diffusion coefficient 4.5 mm$^2$ s$^{-1}$ giving the concentration of repellent compounds throughout the tube at the time points used for data collection. The relative concentrations throughout the tube increase non-linearly with time.
Future Work

Given the success of many of the isovalerate esters in both the short-term and long-term assays, a variety of other carboxylic acids were used to make other series of esters, including isobutyrates, the unsaturated tiglates and 3,3-dimethylacrylates, and the cyclic cyclopropanecarboxylates and cyclobutane-carboxylates. Heavier carboxylate residues may be useful in improving the longevity of some repellents by reducing the rate of vaporization. In addition to varying the carboxylate moiety, future efforts will work on teasing apart the difference between repellency and potency through determination of the vapor pressure of these repellents.

Acknowledgements

We wish to thank the Iowa State University Chemical Instrumentation Facility staff for training and maintenance of the NMR spectrometers used for the chemical characterization of the compounds synthesized for this publication. We would also like to thank the Iowa Agricultural Experimental Station for partial funding of this project.

References


4. Rice, P. J.; Coats, J. R., Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 1994, 87, 1172-1179.


Appendix

The diffusion equation is a partial differential equation, typically written as

\[
\frac{\partial \phi(r, t)}{\partial t} = \nabla \cdot \left[ D(\phi, r) \nabla \phi(r, t) \right],
\]

(1)

where \( \phi(r, t) \) is the concentration of the diffusing material at location \( r \) and time \( t \), \( D(\phi, r) \) is the collective diffusion coefficient for a concentration \( \phi \) at location \( r \), and \( \nabla \) is the vector differential operator del. In the case of a constant diffusion rate \( D \), equation 1 becomes

\[
\frac{\partial \phi(r, t)}{\partial t} = D \nabla^2 \phi(r, t),
\]

(2)

which is equivalent to the heat equation.

In the case of the spatial repellency assay, the system of interest involves an open tube of approximately 300 mm in length with an outer diameter of 90 mm.\(^*\) A single run of the assay involves wetting a 90 mm diameter filter paper with 1 mL of a 0.5\% (w/v) of the solution of interest in acetone;\(^†\) the solution is applied evenly to the filter paper at a rate such that evaporation of acetone occurs readily enough to prevent any solution dripping from the filter paper. After allowing residual acetone to evaporate, the filter paper is placed into a petri dish with an inner diameter of slightly over 90 mm; this allows for a tight fit to the end of the tube. On the other end of the tube, a control filter paper is likewise fitted into a petri dish and attached to the tube. Halfway along the length of the tube is a hole, approximately 25 mm in diameter, through which 25 anesthetized mosquitoes are added; the hole is quickly sealed with tape after the addition of mosquitoes. This produces a closed system with minimal air disturbances.

\(^*\) This gives an inner diameter of approximately 85 mm.
\(^†\) This equates to 5 mg of active compound applied to the filter paper, or roughly 79 \( \mu \)g cm\(^{-2}\) over the whole paper.
As the system is sealed and free of outside influence, the transport of vapors from the repellent is mostly controlled by the rate of diffusion through the tube. Because the treated end uses a filter paper capable of transecting the entire cross-section of the tube, and because the vapor pressures of the compounds tested are low, producing negligible changes in air density upon evaporation, we can model the repellency assay with a 1-dimensional diffusion equation, using the distance $x$ from the treated end to reduce equation 2 to

$$\frac{\partial \phi(x,t)}{\partial t} = D \frac{\partial^2 \phi(x,t)}{\partial x^2}. \quad (3)$$

For the initial conditions, we assume $\phi(x,0) = 0$ for all $x > 0$, and $\phi(0,0) = C_0$, where $C_0$ is a constant calculated from the vapor pressure and molecular weight of the compound being tested. Moreover, we assume that the concentration at the treated filter paper remains constant throughout the entire assay, as the mass of vapor emitted from the filter paper is small compared to the total mass present on the paper, so $\phi(0,t) = C_0$ for all $t \geq 0$. At the untreated end, we have the boundary condition $\frac{\partial \phi(L,t)}{\partial x} = 0$, where $L = 300$ mm is the length of the tube, since the end is sealed and no diffusion occurs through this end point.

These boundary conditions result in an inhomogeneous partial differential equation, which can be solved analytically using Fourier series. The PDE can be written succinctly as

$$u_t = Du_{xx}, \quad 0 < x < L, \quad t > 0$$

BC: $u(0,t) = u_0, \quad u_x(L,t) = 0, \quad t > 0$

IC: $u(x,0) = g(x)$

We note the steady-steady solution is $u_\infty(x) = u_0$, and we can write $u(x,t) = v(x,t) + u_0$. Then

$$u_t = Du_{xx} \Rightarrow v_t = Dv_{xx}$$
\[ u(0, t) = u_0 \Rightarrow u_0 = v(0, t) + u_0 \Rightarrow v(0, t) = 0 \]

\[ u_x(L, t) = 0 \Rightarrow v_x(L, t) = 0 \]

\[ u(x, 0) = v(x, 0) + u_0 \Rightarrow v(x, 0) = g(x) - u_0, \]

and we get the homogeneous PDE

\[ v_t = D v_{xx}, \quad 0 < x < L, \quad t > 0 \]

BC: \[ v(0, t) = 0, \quad v_x(L, t) = 0, \quad t > 0 \]

IC: \[ v(x, 0) = g(x) - u_0. \]

Using separation of variables, we get

\[ v(x, t) = X(x)T(t) \]

\[ T''(t) = -D\lambda^2 T(t) \]

\[ \Rightarrow T(t) = c e^{-D\lambda^2 t} \]

\[ X''(x) = -D\lambda^2 X(x) \]

\[ \Rightarrow X(x) = A \cos(\lambda x) + B \sin(\lambda x) \]

\[ \Rightarrow X'(x) = -A\lambda \sin(\lambda x) + B\lambda \cos(\lambda x) \]

Since \( X(0) = 0 = A, \)

\[ X'(L) = B\lambda \cos(\lambda x) \]

\[ \Rightarrow \lambda_k = \frac{(2k - 1)\pi}{2L}, \quad k = 1, 2, 3, \ldots \]

By the principle of superposition, we get the Fourier series

\[ v(x, t) = \sum_{k=1}^{\infty} B_k e^{-D\lambda^2 t} \sin \left( \frac{(2k - 1)\pi x}{2L} \right). \]

Since

\[ v(x, 0) = \sum_{k=1}^{\infty} B_k \sin \left( \frac{(2k - 1)\pi x}{2L} \right) = g(x) - u_0, \]
we get

\[ B_k = \frac{2}{L} \int_0^L (g(x) - u_0) \sin \left( \frac{(2k - 1)\pi x}{2L} \right) \, dx. \]

In the idealized bioassay setup, \( g(x) = 0 \), so

\[ B_k = -\frac{2u_0}{L} \int_0^L \sin \left( \frac{(2k - 1)\pi x}{2L} \right) \, dx \]

\[ = -\frac{2u_0}{L} \left[ \frac{-2L}{(2k - 1)\pi} \cos \left( \frac{(2k - 1)\pi x}{2L} \right) \right]_0^L \]

\[ = \frac{4u_0}{(2k - 1)\pi} \left( \cos \left( \frac{(2k - 1)\pi}{2} \right) - 1 \right) \]

\[ = -\frac{4u_0}{(2k - 1)\pi}. \]

Thus, for the case \( g(x) = 0 \), we have

\[ u(x, t) = u_0 - \frac{4u_0}{\pi} \sum_{k=1}^{\infty} \frac{e^{-D\lambda^2 t}}{(2k - 1)} \sin \left( \frac{(2k - 1)\pi x}{2L} \right) \]

\[ = u_0 - \frac{2u_0}{L} \sum_{k=1}^{\infty} \lambda^{-1} e^{-D\lambda^2 t} \sin(\lambda x), \]

where \( \lambda = \frac{(2k - 1)\pi}{2L} \).

Unfortunately, this method only works for simple initial conditions, e.g. under the assumption that the concentration of repellent is zero at all points in the chamber at \( t = 0 \).

For the purposes of modelling for this project, \texttt{pdepe}, the initial-boundary conditions solver for parabolic and elliptic PDEs method implemented in MATLAB was used to produce numerical solutions. A mesh with 150 evenly-spaced \( x \)-points were used, and the evaluation of the PDE was performed at 150 evenly-spaced times, over the range of 0 to 150 minutes.
CHAPTER 3. ESTERS OF CITRONELLOL AND CITRONELLIC ACID AS MOSQUITO REPELLENTS

James S. Klimavicz,1* Caleb L. Corona,1 and Joel R. Coats1

1Department of Entomology, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to Pest Management Science.

Abstract

Background

Every year, mosquitoes are responsible for the death of hundreds of thousands of people throughout the world, despite decades of fighting against these insects and the diseases they carry. Insect repellents remain one of the best ways to prevent mosquito bites, and remain important as many mosquito strains develop resistance to common insecticides. Because many plants produce secondary metabolites that are frequently repellent to insects, essential oils derived from these plants, and the compounds found within these oils, are often a useful starting point for developing new mosquito repellents. Starting with citronellol, a monoterpenoid found in high concentration in lemongrass (Cymbopogon sp.) and geranium (Pelargonium sp.), we explored the effects of esterification with different carboxylic acids on the repellency of the resultant citronellyl esters, and compared these to esters derived from the related citronellic acid.

Results

A total of 28 different esters from citronellol or citronellic acid were synthesized, and included a variety of branched alkyl, alicyclic, oxygenated, and aromatic/heteroaromatic carboxylates. Many of the citronellyl esters showed high repellency when tested against Culex

---

* JSK designed, synthesized, purified, and characterized all compounds for testing and wrote the manuscript, CLC performed the bioassay testing. JRC assisted with project formulation and development, and assisted with the design of repellents and revision of the manuscript.
pipiens in a static air repellency chamber using a short-term assay, particularly those with lower molecular weight. However, as expected, these volatile compounds were less effective as long-term repellents. Several of the citronellate esters, derived from citronelic acid, including \( t \)-butyl citronellate and 2-hydroxyethyl citronellate, provided up to 70% repellency even after 7 hours.

**Conclusion**

Although most of the synthetic esters generated moderate-to-good repellency in the short-term bioassay, many of these esters were significantly less successful as long-term repellents. However, in several cases, substantial differences in repellency between similar structures suggests that the choice of the esterifying moiety is important beyond the obvious effect of modulating volatility for achieving potent spatial repellents.

**Keywords:** repellents, mosquito, terpenoids, citronellol esters, biorational

**Introduction**

Mosquito-vectored diseases are responsible for a tremendous health and economic burden throughout the world.\(^1\)\(^-\)\(^2\) While the prevalence of mosquitoes can be reduced through the use of insecticides, gene drives, interference RNA, endosymbiotic bacteria, and biological control methods,\(^3\)\(^-\)\(^6\) repellents remain crucial for the prevention of mosquito bites.\(^7\) Plant essential oils have long been touted for used as arthropod repellents, and while these oils do not provide prolonged protection when compared to synthetic repellents like diethyl 3-toluamide (DEET),\(^8\)\(^-\)\(^9\) many of the natural monoterpenoids and phenylpropanoids found in these oils do possess insecticidal and repellent properties against mosquitos and other insects.\(^10\)\(^-\)\(^11\) Plant essential oils on their own often face substantial hurdles as topical insect repellents. Many of the most repellent compounds are volatile, resulting in short protection times—while DEET-containing products frequently offer protection for six or more hours, many plant essential oils repellent
mosquitoes for fewer than thirty minutes.\textsuperscript{12-14} Additionally, essential oils have strong odors and oily textures that may be off-putting, reducing their utility as a repellent.\textsuperscript{13, 15}

For example, geraniol, geranial, eugenol, citronellol, and citronellal were tested as repellents and fumigants against the cowpea weevil \textit{Callosobruchus maculatus} (Coleoptera: Bruchidae) and the maize weevil \textit{Sitophilus zeamais} (Coleoptera: Curculionidae); in this case, the aldehydes geranial and citronellal were more toxic than the analogous alcohols.\textsuperscript{16} Efficacy of six different monoterpenoids was reported against the Formosan subterranean termite \textit{Coptotermes formosanus} (Isoptera: Rhinotermitidae), a pest with significant economical concern.\textsuperscript{17}

As an alternative to the more-volatile monoterpenoids, terpenoid esters have long been explored as insect repellents or insecticides,\textsuperscript{18} though there are relatively few studies exploring large series of compounds. In one such examination, acetate, propionate, pivalate, trifluoracetate, trichloroacetate, and several other haloacetate esters of seven different monoterpenoids were previously synthesized and screened for topical, fumigant, and ovicidal toxicity in the house fly \textit{Musca domestica} (Diptera: Muscidae) and the red flour beetle, \textit{Tribolium castaneum} (Coleoptera: Tenebrionidae);\textsuperscript{19} in this study, acetates and pivalates were frequently more toxic than the parent monoterpenoid. Additionally, a series of thymyl esters has recently been synthesized and investigated for insecticidal properties against \textit{Spodoptera litura} (Lepidoptera: Noctuidae).\textsuperscript{20}

A previous study of ours looked at isovalerate esters of 21 different monoterpenoids and phenylpropanoids as long-lasting mosquito repellents,\textsuperscript{21} with several monocyclic monoterpenoids provided substantial repellency against mosquitoes for at least seven hours. In the long-term mosquito repellency assay performed with these compounds, citronellyl
isovalerate provided moderate repellency against *Culex pipiens* over a seven-to-eight-hour time period. As a continuation of our structure-activity relation inquiries around monoterpenoid ester repellents, we selected to synthesize a series of citronellol-based esters to explore the effects of modifying the carboxylate residue in these esters.

**Materials and Methods**

**General information**

Citronellol was purchased from Sigma-Aldrich. 4-(Dimethylamino)pyridine (DMAP) and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Chem-Impex (Wood Dale, IL, USA). HPLC-grade dichloromethane (DCM) was purchased from Fisher Scientific. 1-Methylcyclobutanecarboxylic acid was synthesized according to a literature procedure; all other carboxylic acids were purchased from Matrix Scientific (Columbia, SC, USA), Chem-Impex, Oakwood Products (Estill, SC, USA), Synthonix (Wake Forest, NC, USA), or Sigma-Aldrich, and were used as received. The characterization of all compounds was performed at the Iowa State University Chemical Instrumentation Facility. NMR spectra were obtained using Varian MR 400 MHz and Avance III 600 MHz spectrometers. Chemical shifts are reported in ppm relative to the residual solvent peak (CDCl$_3$: 7.26 ppm for $^1$H and 77.16 ppm for $^{13}$C; DMSO-$d_6$: 2.50 for $^1$H and 39.52 ppm for $^{13}$C).

**Citronellyl and citronellate ester synthesis**

Repellent esters were synthesized using the Steglich esterification. Briefly, for the citronellyl carboxylates, citronellol (781 mg, 5 mmol) was dissolved in dichloromethane (DCM, 25 mL), and carboxylic acid (6 mmol) and DMAP (61.1 mg, 0.5 mmol) were added. The solution was cooled to 0 °C, and DCC (5.25 mmol, 1.08 g) was added portion-wise. For alkyl citronelllates, citronellic acid (851 mg, 5 mmol) was dissolved in DCM (25 mL), and an alcohol (6 mmol) and DMAP (61.1 mg, 0.5 mmol) were added. The solution was cooled to 0 °C, and
DCC (5.25 mmol, 1.08 g) was added portion-wise. In either reaction, after the addition of DCC, the reaction was stirred at 0 °C for 15 minutes, and then warmed to 22 °C for 2 hours. Hexane (75 mL) was added, and the white precipitate of 1,3-dicyclohexylurea was removed by filtration. The filtrate was washed with water (50 mL), 1 M hydrochloric acid (50 mL) and 1 M sodium hydroxide (50 mL), and then dried over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the crude ester was purified by column chromatography using 9:1 hexane:ethyl acetate as eluent.

**Citronellyl formate (1a)** Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.05 (s, 1H), 5.08 (tp, $J$ = 7.1, 1.4 Hz, 1H), 4.28 – 4.14 (m, 2H), 2.08 – 1.88 (m, 2H), 1.75 – 1.66 (m, 1H), 1.68 (d, 1H), 1.60 (d, $J$ = 1.6 Hz, 3H), 1.56 (dtt, $J$ = 13.2, 6.6, 1.3 Hz, 1H), 1.47 (dt, $J$ = 13.7, 7.6, 6.0 Hz, 1H), 1.35 (dddd, $J$ = 13.5, 9.4, 6.5, 5.6 Hz, 1H), 1.19 (dddd, $J$ = 13.5, 9.5, 7.8, 5.8 Hz, 1H), 0.92 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 161.3, 131.6, 124.6, 62.6, 37.1, 35.5, 25.8, 25.5, 19.4, 17.8.

**Citronellyl isobutyrate (1b)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.07 (thept, $J$ = 7.1, 1.4 Hz, 1H), 4.15 – 4.00 (m, 2H), 2.52 (hept, $J$ = 7.0 Hz, 1H), 2.07 – 1.87 (m, 2H), 1.67 (d, 1H), 1.70 – 1.61 (m, 1H), 1.59 (d, $J$ = 1.4 Hz, 3H), 1.53 (dddd, $J$ = 13.1, 6.5, 3.8, 1.2 Hz, 1H), 1.48 – 1.38 (m, 1H), 1.34 (dddd, $J$ = 12.0, 9.2, 5.9, 4.7 Hz, 1H), 1.23 – 1.13 (m, 1H) 1.15 (d, $J$ = 7.0 Hz, 6H), 0.90 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.4, 131.5, 124.7, 62.9, 37.1, 35.6, 34.2, 29.6, 25.9, 25.5, 19.6, 19.1, 17.8.

**Citronellyl isovalerate (1c)** Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 5.08 (thept, $J$ = 7.1, 1.4 Hz, 1H), 4.20 – 4.03 (m, 2H), 2.17 (d, $J$ = 7.1 Hz, 2H), 2.09 (thept, $J$ = 7.5, 6.5 Hz, 1H), 2.04 – 1.90 (m, 2H), 1.68 (d, $J$ = 1.4 Hz, 3H), 1.71 – 1.62 (m, 1H), 1.60 (d, $J$ = 1.4 Hz, 3H), 1.43 (dt, $J$ = 13.7, 7.6, 6.0 Hz, 1H), 1.34 (dddd, $J$ = 13.3, 9.6, 6.4, 5.5 Hz, 1H), 1.18 (dddd, $J$ = 13.5, 9.5, 7.8, 5.8 Hz, 1H), 0.92 (d, $J$ = 6.6 Hz, 3H).
9.5, 7.8, 5.8 Hz, 1H), 0.95 (d, J = 6.6 Hz, 6H), 0.91 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 173.4, 131.5, 124.7, 62.8, 43.7, 37.1, 35.7, 29.6, 25.89, 25.85, 25.5, 22.5, 19.5, 17.8, 14.3.

**Citronellyl pivalate** (1d) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) 5.08 (thept, J = 7.1, 1.4 Hz, 1H), 4.13 – 4.03 (m, 2H), 2.04 – 1.94 (m, 2H), 1.75 – 1.66 (m, 1H), 1.67 (d, J = 1.4 Hz, 3H), 1.59 (d, J = 1.5 Hz, 3H), 1.55 (dtt, J = 13.2, 6.6, 1.3 Hz, 1H), 1.42 (dtd, J = 13.6, 7.6, 6.0 Hz, 1H), 1.34 (dddt, J = 13.5, 9.4, 6.5, 5.6 Hz, 1H), 1.22 – 1.14 (m, 1H), 1.19 (s, 9H) f0.92 (d, J = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.8, 131.4, 124.7, 62.9, 38.8, 37.1, 35.6, 29.6, 27.3, 25.8, 25.5, 19.6, 17.8.

**Citronellyl levulinate** (1e) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ thept (tp, J = 7.2, 1.5 Hz, 1H), 4.21 – 3.98 (m, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.56 (t, J = 6.6 Hz, 2H), 2.19 (s, 3H), 2.04 – 1.88 (m, 2H), 1.67 (d, J = 1.6 Hz, 3H), 1.70 – 1.61 (m, 1H), 1.59 (d, J = 1.4 Hz, 3H), 1.57 – 1.48 (m, 1H), 1.49 – 1.37 (m, 1H), 1.33 (dddt, J = 13.4, 12.0, 7.1, 3.1 Hz, 1H), 1.17 (dddt, J = 13.6, 9.5, 7.7, 6.1 Hz, 1H), 0.90 (d, J = 6.5 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 206.8, 173.0, 131.5, 124.7, 63.4, 38.1, 37.1, 35.5, 30.0, 29.6, 28.1, 25.9, 25.5, 19.5, 17.8.

**Citronellyl difluoroacetate** (1f) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.89 (t, J = 53.4 Hz, 1H), 5.07 (thept, J = 7.1, 1.4 Hz, 1H), 4.40 – 4.25 (m, 2H), 2.08 – 1.88 (m, 1H), 1.80 – 1.70 (m, 1H), 1.68 (d, J = 1.4 Hz, 3H), 1.60 (d, J = 1.3 Hz, 3H), 1.58 – 1.47 (m, 2H), 1.34 (dddt, J = 15.6, 11.5, 7.8, 5.8 Hz, 2H), 1.20 (dddt, J = 13.5, 9.3, 7.5, 6.1 Hz, 1H), 0.93 (d, J = 6.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 162.6 (t, J = 28.6 Hz), 131.6, 124.3, 106.7 (t, J = 249.3 Hz), 77.3, 77.0, 76.7, 65.4, 36.8, 35.0, 29.2, 25.7, 25.3, 19.3, 17.6. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -126.64 (d, J = 53.4 Hz).
Citronellyl tiglate (1g) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.83 (qq, $J = 7.0, 1.4$ Hz, 1H), 5.08 (tp, $J = 7.1, 1.4$ Hz, 1H), 4.23 – 4.09 (m, 2H), 2.08 – 1.88 (m, 2H), 1.82 (dq, $J = 1.4, 1.2$ Hz, 3H), 1.78 (dq, $J = 7.0, 1.2$ Hz, 3H), 1.75 – 1.69 (m, 1H), 1.67 (d, $J = 1.4$ Hz, 3H), 1.61 – 1.53 (m, 1H), 1.60 (d, $J = 1.3$ Hz, 3H), 1.46 (ddt, $J = 13.3, 7.7, 6.4$ Hz, 1H), 1.36 (dddd, $J = 13.3, 9.3, 6.5, 5.5$ Hz, 1H), 1.19 (dddd, $J = 13.6, 9.2, 7.6, 6.2$ Hz, 1H), 0.92 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.4, 137.0, 131.5, 128.9, 124.7, 63.1, 37.1, 35.6, 29.7, 25.8, 25.5, 19.6, 17.8, 14.5, 12.2.

Citronellyl 3,3-dimethylacrylate (1h) Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 5.66 (s, 1H), 5.08 (thept, $J = 7.3, 1.5$ Hz, 1H), 4.17 – 4.06 (m, 2H), 2.16 (s, 3H), 1.99 (ddp, $J = 29.3, 14.9, 8.2, 7.4$ Hz, 2H), 1.88 (s, 3H), 1.71 – 1.65 (m, 1H), 1.67 (s, 3H), 1.59 (s, 3H), 1.59 – 1.52 (m, 1H), 1.45 (dt, $J = 15.1, 6.6$ Hz, 1H), 1.35 (dddd, $J = 13.5, 9.3, 6.4, 5.4$ Hz, 1H), 1.18 (dddd, $J = 13.6, 9.3, 7.6, 6.0$ Hz, 1H), 0.91 (dd, $J = 6.7, 1.9$ Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.0, 156.4, 131.4, 124.8, 116.3, 62.2, 37.2, 35.7, 29.7, 27.5, 25.8, 25.5, 20.3, 19.6, 17.8.

Citronellyl cyclopropanecarboxylate (2a) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.09 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.17 – 4.02 (m, 2H), 2.08 – 1.88 (m, 2H), 1.71 – 1.65 (m, 1H), 1.68 (d, $J = 1.3$ Hz, 3H), 1.63 – 1.52 (m, 2H), 1.60 (d, $J = 1.5$ Hz, 3H), 1.48 – 1.40 (m, 1H), 1.35 (dddd, $J = 13.5, 9.4, 6.5, 5.5$ Hz, 1H), 1.18 (dddd, $J = 13.6, 9.4, 7.6, 6.1$ Hz, 1H), 1.01 – 0.95 (m, 2H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.87 – 0.80 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 175.1, 131.5, 124.7, 63.1, 37.1, 35.6, 29.6, 25.9, 25.5, 19.6, 17.8, 13.1, 8.4.

Citronellyl cyclobutanecarboxylate (2b) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.08 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.17 – 4.03 (m, 2H), 3.11 (pd, $J = 8.5, 1.1$ Hz, 1H), 2.36 – 2.23 (m, 2H), 2.22 – 2.11 (m, 1H), 2.07 – 1.81 (m, 4H), 1.71 – 1.65 (m, 1H), 1.67 (d, $J = 1.5$ Hz, 3H), 1.60 (d, $J = 1.3$ Hz, 3H), 1.57 – 1.49 (m, 1H), 1.48 – 1.39 (m, 1H), 1.34 (dddd, $J = 13.3, 9.3, 6.5,$
5.4 Hz, 1H), 1.18 (dddd, \(J = 13.5, 9.2, 7.6, 6.1\) Hz, 1H), 0.91 (d, \(J = 6.6\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 175.7, 131.5, 124.7, 62.9, 38.3, 37.1, 35.6, 29.6, 25.8, 25.5, 25.41, 25.39, 19.6, 18.5, 17.8.

**Citronellyl cyclopentanecarboxylate (2c)** Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.08 (thept, \(J = 7.1, 1.4\) Hz, 1H), 4.28 – 3.93 (m, 3H), 2.71 (tt, \(J = 8.6, 7.4\) Hz, 1H), 2.07 – 1.91 (m, 2H), 1.90 – 1.82 (m, 2H), 1.81 – 1.73 (m, 2H), 1.72 – 1.63 (m, 3H), 1.67 (d, \(J = 1.3\) Hz, 3H), 1.61 – 1.50 (m, 3H), 1.60 (d, \(J = 1.4\) Hz, 3H), 1.48 – 1.40 (m, 1H), 1.40 – 1.29 (m, 1H), 1.18 (dddd, \(J = 13.5, 9.2, 7.7, 6.1\) Hz, 1H), 0.91 (d, \(J = 6.6\) Hz, 4H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.0, 131.5, 124.7, 62.9, 44.1, 37.1, 35.6, 30.14, 30.13, 29.6, 25.9, 25.8, 25.5, 19.6, 17.8.

**Citronellyl cyclohexanecarboxylate (2d)** Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.08 (thept, \(J = 7.2, 1.5\) Hz, 1H), 4.16 – 4.01 (m, 2H), 2.27 (tt, \(J = 11.3, 3.6\) Hz, 1H), 2.05 – 1.92 (m, 2H), 1.92 – 1.85 (m, 3H), 1.78 – 1.70 (m, 2H), 1.67 (d, \(J = 1.4\) Hz, 3H), 1.69 – 1.60 (m, 2H), 1.53 (dt, \(J = 12.9, 6.7\) Hz, 1H), 1.48 – 1.36 (m, 3H), 1.35 – 1.21 (m, 5H), 1.17 (dddd, \(J = 13.6, 9.1, 7.5, 6.1\) Hz, 1H), 0.90 (d, \(J = 6.6\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.4, 131.5, 124.7, 62.7, 43.4, 37.1, 35.6, 29.5, 29.2, 25.9, 25.8, 25.6, 25.5, 19.6, 17.8.

**Citronellyl 3-methylene cyclobutanecarboxylate (3a)** Colorless oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 5.08 (thept, \(J = 7.3, 1.5\) Hz, 1H), 4.80 (p, \(J = 2.4\) Hz, 2H), 4.19 – 4.08 (m, 2H), 3.15 – 3.06 (m, 1H), 3.03 – 2.95 (m, 2H), 2.93 – 2.85 (m, 2H), 1.96 (tq, \(J = 14.5, 7.4\) Hz, 2H), 1.71 – 1.65 (m, 1H), 1.68 (s, 3H), 1.60 (s, 3H), 1.54 (dq, \(J = 12.7, 6.1\) Hz, 1H), 1.44 (dq, \(J = 14.2, 7.2\) Hz, 1H), 1.34 (dddd, \(J = 13.4, 9.3, 6.5, 5.4\) Hz, 1H), 1.18 (dddd, \(J = 13.6, 9.3, 7.7, 6.1\) Hz, 1H), 0.91 (d, \(J = 6.5\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 175.3, 144.6, 131.5, 124.7, 106.9, 63.3, 37.1, 35.60, 35.57, 35.55f, 33.4, 29.6, 25.9, 25.5, 19.6, 17.8.
Citronellyl 3,3-difluorocyclobutanecarboxylate (3b) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.07 (thept, $J = 7.1, 1.5$ Hz, 1H), 4.23 – 4.08 (m, 2H), 2.99 – 2.89 (m, 1H), 2.89 – 2.73 (m, 4H), 2.07 – 1.89 (m, 2H), 1.71 – 1.67 (m, 1H), 1.68 (s, 3H), 1.60 (s, 3H), 1.58 – 1.49 (m, 1H), 1.49 – 1.40 (m, 1H), 1.34 (dddd, $J = 14.7, 9.3, 6.5, 5.4$ Hz, 1H), 1.19 (dddd, $J = 13.6, 9.2, 7.6, 6.1$ Hz, 1H), 0.91 (d, $J = 6.5$ Hz, 3H). $^{19}$F NMR (376 MHz, CDCl$_3$) δ -82.77 (dttd, $J = 191.8, 11.6, 6.4, 3.3$ Hz, 1F), -97.17 (dttd, $J = 192.8, 16.8, 13.9, 2.5$ Hz, 1F). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 173.5 (t, $J = 2.6$ Hz), 131.6, 124.6, 118.9 (dd, $J = 284.2, 270.8$ Hz), 64.0, 38.9, 37.1, 35.5, 38.9 (dd, $J = 25.8, 24.2$ Hz), 38.7 (dd, $J = 26.1, 24.5$ Hz), 29.5, 26.7 (dd, $J = 14.3, 5.2$ Hz), 25.9, 25.5, 19.5, 17.8.

Citronellyl 3-oxocyclobutanecarboxylate (3c) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.07 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.28 – 4.05 (m, 2H), 3.46 – 3.36 (m, 2H), 3.33 – 3.27 (m, 1H), 3.26 – 3.15 (m, 2H), 2.07 – 1.87 (m, 2H), 1.73 – 1.67 (m, 1H), 1.67 (d, $J = 1.7$ Hz, 3H), 1.59 (s, 3H), 1.54 (ddt, $J = 12.8, 6.4, 1.2$ Hz, 1H), 1.46 (dtd, $J = 13.5, 7.3, 5.9$ Hz, 1H), 1.39 – 1.29 (m, 1H), 1.19 (dddd, $J = 15.0, 9.2, 7.4, 3.6$ Hz, 1H), 0.92 (dd, $J = 6.4, 1.2$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 203.9, 174.2, 131.6, 124.5, 64.0, 51.7, 37.0, 35.5, 29.5, 27.6, 25.8, 25.5, 19.5, 17.8.

Citronellyl 1-methylcyclobutanecarboxylate (3d) Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) δ 5.08 (thept, $J = 7.3, 1.5$ Hz, 1H), 4.16 – 4.06 (m, 2H), 2.45 (dd, $J = 13.6, 5.8$ Hz, 2H), 2.05 – 1.88 (m, 3H), 1.87 – 1.77 (m, 2H), 1.76 – 1.70 (m, 1H), 1.67 (s, 3H), 1.60 (s, 3H), 1.57 – 1.53 (m, 1H), 1.44 (dt, $J = 13.6, 6.3$ Hz, 1H), 1.39 (s, 3H), 1.37 – 1.23 (m, 3H), 1.19 (ddt, $J = 11.6, 7.7, 4.8$ Hz, 1H), 0.91 (dd, $J = 6.6, 1.8$ Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 178.1, 131.5, 124.7, 63.0, 43.6, 37.1, 35.6, 31.64, 31.63, 29.6, 25.8, 25.5, 24.1, 19.6, 17.8, 15.4.
Citronellyl benzoate (4a) Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.08 – 8.01 (m, 2H), 7.60 – 7.51 (m, 1H), 7.48 – 7.39 (m, 2H), 5.10 (thept, \(J = 7.1, 1.4\) Hz, 1H), 4.45 – 4.23 (m, 2H), 2.11 – 1.91 (m, 2H), 1.82 (dtd, \(J = 13.4, 7.1, 5.0\) Hz, 1H), 1.71 – 1.64 (m, 1H), 1.67 (d, \(J = 1.4\) Hz, 3H), 1.62 – 1.54 (m, 1H), 1.60 (d, \(J = 1.3\) Hz, 3H), 1.41 (ddddd, \(J = 13.4, 9.4, 6.5, 5.4\) Hz, 1H), 1.24 (ddddd, \(J = 13.6, 9.2, 7.6, 6.2\) Hz, 1H), 0.97 (d, \(J = 6.5\) Hz, 6H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 166.8, 132.9, 131.5, 130.6, 129.7, 128.4, 124.7, 63.6, 37.1, 35.6, 29.7, 25.8, 25.5, 19.6, 17.8.

Citronellyl 2-furoate (4b) Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.57 (dd, \(J = 1.7, 0.9\) Hz, 1H), 7.15 (dd, \(J = 3.5, 0.8\) Hz, 1H), 6.50 (dd, \(J = 3.5, 1.8\) Hz, 1H), 5. (thept, \(J = 7.3, 1.5\) Hz, 1H), 4.41 – 4.26 (m, 2H), 2.09 – 1.89 (m, 2H), 1.79 (dt, \(J = 13.2, 7.2, 4.6\) Hz, 1H), 1.67 (q, \(J = 1.3\) Hz, 3H), 1.65 – 1.60 (m, 1H), 1.59 (d, \(J = 1.3\) Hz, 3H), 1.58 – 1.49 (m, 1H), 1.38 (ddddd, \(J = 13.4, 9.4, 6.5, 5.3\) Hz, 1H), 1.21 (ddddd, \(J = 13.6, 9.3, 7.5, 6.1\) Hz, 1H), 0.95 (d, \(J = 6.5\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 159.0, 146.3, 145.0, 131.5, 124.6, 117.8, 111.9, 63.7, 37.1, 35.6, 29.6, 25.8, 25.5, 19.6, 17.8.

Citronellyl 3-furoate (4c) Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.99 (dd, \(J = 1.6, 0.8\) Hz, 1H), 7.42 (t, \(J = 1.7\) Hz, 1H), 6.73 (dd, \(J = 1.9, 0.8\) Hz, 1H), 5.09 (thept, \(J = 7.1, 1.4\) Hz, 1H), 4.33 – 4.22 (m, 2H), 2.10 – 1.90 (m, 2H), 1.82 – 1.68 (m, 1H), 1.71 – 1.65 (m, 1H), 1.67 (d, \(J = 1.3\) Hz, 3H), 1.60 (s, 3H), 1.58 – 1.47 (m, 1H), 1.38 (ddddd, \(J = 13.3, 9.3, 6.5, 5.4\) Hz, 1H), 1.22 (ddddd, \(J = 13.6, 9.3, 7.5, 6.2\) Hz, 1H), 0.95 (d, \(J = 6.5\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 163.3, 147.7, 143.8, 131.5, 124.7, 119.8, 110.0, 63.2, 37.1, 35.6, 29.7, 25.8, 25.5, 19.6, 17.8.

Citronellyl 2-thiophenecarboxylate (4d) Colorless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.79 (dd, \(J = 3.7, 1.3\) Hz, 1H), 7.54 (dd, \(J = 5.0, 1.3\) Hz, 1H), 7.09 (dd, \(J = 5.0, 3.7\) Hz, 1H), 5.09
(thept, $J = 7.1, 1.4$ Hz, 1H), 4.40 – 4.26 (m, 2H), 2.10 – 1.90 (m, 2H), 1.79 (dtd, $J = 13.3, 7.0, 4.8$ Hz, 1H), 1.71 – 1.65 (m, 1H), 1.67 (d, $J = 1.3$ Hz, 3H), 1.60 (s, 3H), 1.59 – 1.50 (m, 1H), 1.39 (ddddd, $J = 13.3, 9.3, 6.6, 5.4$ Hz, 1H), 1.22 (ddddd, $J = 13.6, 9.3, 7.5, 6.2$ Hz, 1H), 0.96 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 162.5, 134.3, 133.4, 132.3, 131.5, 127.8, 124.7, 63.8, 37.1, 35.6, 29.7, 25.8, 25.5, 19.6, 17.8.

Citronellyl 3-thiophenecarboxylate (4e) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09 (dd, $J = 3.1, 1.2$ Hz, 1H), 7.52 (dd, $J = 5.1, 1.2$ Hz, 1H), 7.30 (dd, $J = 5.1, 3.1$ Hz, 1H), 5.09 (thept, $J = 7.3, 1.4$ Hz, 1H), 4.38 – 4.25 (m, 2H), 2.11 – 1.90 (m, 2H), 1.79 (dtd, $J = 13.4, 7.1, 4.9$ Hz, 1H), 1.71 – 1.64 (m, 1H), 1.67 (d, $J = 1.4$ Hz, 3H), 1.60 (s, 3H), 1.54 (dddd, $J = 13.2, 7.9, 6.5$ Hz, 1H), 1.39 (ddddd, $J = 13.3, 9.3, 6.5, 5.3$ Hz, 1H), 1.23 (ddddd, $J = 13.5, 9.2, 7.6, 6.2$ Hz, 1H), 0.96 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 163.0, 134.2, 132.6, 131.5, 128.1, 126.0, 124.7, 63.4, 37.1, 35.6, 29.7, 25.8, 25.5, 19.6, 17.8.

Citronellyl thiophen-2-ylacetate (4f) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.21 (dd, $J = 5.0, 1.4$ Hz, 1H), 6.99 – 6.91 (m, 2H), 5.08 (tp, $J = 7.1, 1.4$ Hz, 1H), 4.29 – 4.07 (m, 2H), 3.83 (d, $J = 0.9$ Hz, 2H), 2.11 – 1.85 (m, 2H), 1.69 (d, $J = 1.4$ Hz, 3H), 1.73 – 1.64 (m, 1H), 1.60 (d, $J = 1.3$ Hz, 3H), 1.53 (ddtd, $J = 12.7, 6.3, 5.3, 1.0$ Hz, 1H), 1.45 (ddddd, $J = 13.2, 7.9, 6.9, 6.0$ Hz, 1H), 1.33 (ddddd, $J = 13.3, 9.5, 6.5, 5.3$ Hz, 1H), 1.17 (ddddd, $J = 13.6, 9.4, 7.7, 6.0$ Hz, 1H), 0.90 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 170.7, 135.3, 131.5, 126.9, 125.1, 124.7, 63.9, 37.1, 35.7, 35.5, 29.5, 25.9, 25.5, 19.5, 17.8.

tert-Butyl citronellate (5a) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.09 (thept, $J = 7.3, 1.5$ Hz, 1H), 2.21 (dd, $J = 14.2, 5.8$ Hz, 1H), 2.01 (dd, $J = 14.2, 8.1$ Hz, 1H), 2.00 – 1.87 (m, 2H), 1.68 (d, $J = 1.6$ Hz, 3H), 1.60 (d, $J = 1.6$ Hz, 3H), 1.44 (s, 9H), 1.33 (ddddd, $J = 16.4, 10.4,$
8.0, 4.9 Hz, 1H), 1.20 (dddd, J = 13.5, 9.2, 7.7, 6.2 Hz, 1H), 0.93 (d, J = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 172.9, 131.6, 124.6, 80.1, 43.3, 36.9, 30.3, 28.3, 25.9, 25.6, 19.7, 17.8.

**1,1-Dimethylallyl citronellate (5b)** Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) δ 6.08 (dd, J = 17.5, 10.9 Hz, 1H), 5.16 (dd, J = 17.5, 0.8 Hz, 1H), 5.09 (thept, J = 7.3, 1.5 Hz, 1H), 5.06 (dd, J = 11.0, 0.9 Hz, 1H), 2.24 (dd, J = 14.6, 6.0 Hz, 1H), 2.04 (dd, J = 14.6, 8.2 Hz, 1H), 2.01 – 1.88 (m, 3H), 1.67 (d, J = 1.3 Hz, 3H), 1.59 (s, 3H), 1.51 (s, 6H), 1.34 (dddd, J = 13.4, 9.5, 6.6, 5.7 Hz, 1H), 1.20 (ddddd, J = 13.5, 9.4, 7.7, 6.0 Hz, 1H), 0.93 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 172.4, 142.9, 131.6, 124.5, 112.6, 80.5, 43.0, 36.9, 30.2, 26.6, 26.6, 25.8, 25.6, 19.7, 17.8.

**1,1-Dimethylpropargyl citronellate (5c)** Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) δ 5.08 (thept, J = 7.3, 1.5 Hz, 1H), 2.51 (s, 1H), 2.27 (dd, J = 14.6, 6.0 Hz, 1H), 2.07 (dd, J = 14.6, 8.2 Hz, 1H), 2.04 – 1.90 (m, 3H), 1.672 (s, 3H), 1.666 (s, 3H), 1.59 (s, 3H), 1.35 (ddt, J = 12.6, 9.4, 6.2 Hz, 1H), 1.22 (ddddd, J = 13.5, 9.3, 7.8, 6.0 Hz, 1H), 0.95 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 171.7, 131.6, 124.5, 85.0, 72.3, 71.5, 42.5, 36.8, 30.2, 29.08, 29.07, 25.8, 25.5, 19.7, 17.8.

**3-Oxetanyl citronellate (5d)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.42 (thept, J = 7.3, 1.5 Hz, 1H), 4.89 (ddd, J = 7.4, 6.4, 0.9 Hz, 3H), 4.63 (ddd, J = 7.5, 5.3, 1.0 Hz, 2H), 2.36 (dd, J = 14.8, 6.0 Hz, 1H), 2.16 (dd, J = 14.8, 8.2 Hz, 1H), 2.09 – 1.89 (m, 3H), 1.68 (d, J = 1.4 Hz, 3H), 1.60 (d, J = 1.4 Hz, 3H), 1.42 – 1.30 (m, 1H), 1.24 (ddddd, J = 13.5, 9.1, 7.8, 6.2 Hz, 1H), 0.96 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 172.7, 131.9, 124.2, 77.8, 67.82, 67.79, 41.5, 36.8, 30.1, 25.9, 25.5, 19.7, 17.8.

**(3-Methyloxetan-3-yl)methyl citronellate (5e)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.08 (thept, J = 7.3, 1.5 Hz, 1H), 4.52 (d, J = 6.0 Hz, 2H), 4.38 (d, J = 5.9 Hz, 2H),
4.16 (s, 2H), 2.36 (dd, J = 14.7, 6.0 Hz, 1H), 2.17 (dd, J = 14.7, 8.2 Hz, 1H), 2.06 – 1.89 (m, 3H), 1.68 (d, J = 1.6 Hz, 3H), 1.60 (d, J = 1.4 Hz, 3H), 1.42 – 1.30 (m, 1H), 1.34 (s, 3H), 1.23 (dddd, J = 13.6, 9.1, 7.8, 6.1 Hz, 1H), 0.95 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 173.5, 131.8, 124.3, 79.7, 68.6, 41.8, 39.2, 36.9, 30.2, 25.9, 25.5, 21.4, 19.8, 17.8.

**2-Hydroxyethyl citronellate (5f)** Colorless oil. $^1$H NMR (600 MHz, CDCl$_3$) δ 5.08 (thept, J = 7.3, 1.5 Hz, 1H), 4.24 – 4.17 (m, 2H), 3.84 – 3.80 (m, 2H), 2.36 (dd, J = 14.8, 5.9 Hz, 1H), 2.16 (dd, J = 14.8, 8.2 Hz, 1H), 2.05 – 1.91 (m, 3H), 1.67 (d, J = 1.5 Hz, 3H), 1.59 (s, 2H), 1.35 (dddd, J = 16.1, 12.8, 9.3, 6.4 Hz, 1H), 1.23 (dddd, J = 13.6, 9.5, 7.9, 5.9 Hz, 1H), 0.95 (d, J = 6.7 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 173.8, 131.8, 124.3, 66.0, 61.5, 41.8, 36.9, 30.1, 25.8, 25.5, 19.7, 17.8.

**Mosquito repellency testing**

The repellency assay used a static air chamber as described in previous work.$^{21, 26}$ For each trial, a 90-mm filter paper (Whatman No. 1) was treated with 1 mL of a 0.5 w/v solution of a citronellyl ester in acetone, giving a nominal repellent concentration of 79 μg cm$^{-2}$ on the filter paper. During the addition of this acetone solution, the filter paper was placed atop several pins supported in a cardboard substrate to minimize transfer of the treatment solution from the filter paper to a surface beneath the paper; additionally, the solution was added to the filter paper as a rate slow enough to prevent dripping off the filter paper, and the acetone was allowed to evaporate in open air for 10 minutes for the short-term repellency assays, or for 5 hours for the long-term assays.

The chamber itself consists of a clear glass cylinder (length: 600 mm; I.D. 85 mm) laying horizontally with a single 20-mm hole in the side of the cylinder centered at 300 mm. One end of the cylinder was covered with a glass petri dish (I.D. 90 mm) containing an untreated 90-mm filter paper, and the petri dish was affixed with tape. The other end is capped in the same
manner, except with the treated filter paper. Immediately after placing the filter papers on the end of the tube, twenty non-blood-fed female *Culex pipiens* mosquitoes were anesthetized with carbon dioxide, and placed into the tube via the 20-mm hole using a funnel, and the hole was sealed after the addition of the mosquitoes. At 15, 30, 60, 90, 120, and 150 minutes after the addition of the mosquitoes to the chamber, the number of mosquitoes on the half of the chamber containing the treated filter paper were counted. Mosquitoes were only used for a single assay.

**Data analysis**

All statistical analysis was performed in R. Percentage repellency ($\%_r$) is calculated as

$$\%_r = 100\% \cdot \frac{n_t - n_u}{n_t + n_u},$$

where $n_t$ and $n_u$ are the number of mosquitoes on the treated and untreated sides of the repellency chamber, respectively. The total number of mosquitoes and the number of mosquitoes on the untreated half of the chamber in each trial at a given time point for a given compound were fit to a beta-binomial distribution before converting to percentage repellency to account for overdispersion in the estimated repellency (see Appendix for details). Because the calculated $\%_r$ is bound between -100% (completely attracting) and 100% (completely repelling), percentage repellency shown with error bars representing 50% confidence intervals derived from the beta-binomial distribution, as opposed to using a normal approximation for estimating standard errors. Statistically significant differences were determined by non-overlap of these confidence intervals.

**Results**

Using the Steglich esterification,$^{24}$ 22 esters were synthesized in good-to-excellent yields from citronellol, and six from citronelic acid. The citronellyl esters can be broadly broken down into four main groups based on structure, as seen in Scheme 3.1. The acyclic esters include not
only the formate (1a), isobutyrate (1b), isovalerate (1c), and pivalate (1d), but also the
oxygenated levulinate (1e), difluoroacetate (1f), and the unsaturated isomeric tiglate (1g) and
3,3-dimethylacrylate (1h) esters. The alicyclic esters include the cyclopropanecarboxylate (2a),
cyclobutanecarboxylate (2b), cyclopentanecarboxylate (2c), and cyclohexanecarboxylate (2d).
Several derivatives of cyclobutanecarboxylic acid were also used, forming the methylene
derivative 3a, the difluorinated 3b, the ketone 3c, and the 1-methyl-1-cyclobutanecarboxylate
derivative 3d. The esters containing aromatic rings included the benzoate (4a), the 2-furate (4b)
and its isomer the 3-furoate (4c), the 2-thiophenecarboxylate (4d) and its isomer 4e, as well as
the (thiophen-2-yl)acetate ester 4f.

Scheme 3.2 shows the six esters synthesized from citronellic acid, also using the Steglich
esterification. The t-butyl ester (5a) is isomeric with 4b, while the 1,1-dimethylallyl ester 5b is
isomeric to both 1g and 1h, and the 1,1-dimethylpropargyl ester 5c was chosen as an analog to
5b. Oxetanes 5d and 5e were synthesized to exam the effects of additional hydrogen-bond-
accepting oxygen atoms in the repellent, while the hydroxyethyl ester 5f contains a hydrogen bond donor.

Of the acyclic esters, the aliphatic esters 1a-d achieved very high repellency in the short-term assay, though the repellency was substantially lower in the longer assay (Figure 3.1). Of these four esters, the citronellyl isovalerate, as assayed in our previous study,21 provided numerically lower repellency in the short-term assay. The unsaturated citronellyl esters 1g and 1h provided significantly lower repellency than most of the aliphatic esters beyond 30 minutes, while the difluoroacetate 1f was also a very potent repellent in the short term. 1e, the levulinate ester, provided modest repellency. In the long-term assay, most of the esters 1a-h provided lower repellency than in the short-term assay as the repellent evaporated off of the filter paper, though 1e performed nearly identically to its short-term assay. Surprisingly, 1g, performed significantly better in the long-term assay than in the short-term assay.

There were substantial differences among the alicyclic citronellyl esters in the short-term bioassay, with the cyclopropanecarboxylate 2a and cyclobutanecarboxylate 2b significantly outperforming the cyclopentanecarboxylate 2c and cyclohexanecarboxylate 2d (Figure 3.2). In
Figure 3.1. Short- and long-term repellency for citronellyl esters 1a-h. Data points are staggered around the time points on the x-axis for clarity.

Figure 3.2. Short- and long-term repellency for alicyclic citronellyl esters 2a-d.
Several derivatives of cyclobutanecarboxylic acid were also used for esterifications to make compounds 3a-d to assess the effects of substitutions on the parent compounds 2b. Over the course of the short-term assay, 2b numerically outperformed 3a-d after 60 minutes (Figure 3.3). Methylene-substituted 3a and the difluorinated 3b provided particularly poor repellency in the short-term and long-term assays. The ketone 3c was numerically slightly better over the short-term, but provided almost no repellency in the long-term assay. The 1-methylcyclobutanecarboxylate 3d was roughly comparable to either 2b or 3c.
The aromatic carboxylate esters 4a-f contain phenyl, furan, or thiophene rings, and provided moderate-to-negligible repellency in both the short- and long-term assays (Error! Reference source not found.). The 2- and 3-furoates, 4b and 4c, respectively, numerically outperformed the 2- and 3- thiophenecarboxylates (4d and 4e) in both time regimes., while citronellyl benzoate (4a) and the thiophen-2-ylacetate 4f offered poor short- and long-term repellency.

Among the citronellate esters 5a-f, the 1,1-dimethylallyl derivative 5b and 2-hydroxyethyl ester 5f provided the best short-term repellency (Figure 3.5). However, 5b offered no repellency at any time point in the long-term assay, while 5f continued to repel mosquitoes. The tert-butyl and 1,1-dimethylpropargyl esters (5a and 5c, respectively), which are structurally
similar to $5b$, provided modest repellency under both assays. The citronellate esters containing oxetane rings ($5e$ and $5f$) were slightly-to-moderately repellent.

**Discussion**

While volatility of a repellent plays a significant role in the comparative repellency of these compounds, it does not explain all of the observed trends. Repellency itself depends on both the concentration of the repellent, as well as the innate potency of the compound. Compounds that are more volatile are expected to have substantially lower concentrations on the treated filter paper in the long-term assay than in the short-term assay when compared to those repellents with lower volatility; however, esters with very low volatility perform poorly in spatial repellency assays unless they are particularly potent. Compounds with similar expected volatilities that have significantly different abilities to repel mosquitoes likely have a considerable difference in potency.

Figure 3.5. Short- and long-term repellency for citronellate esters **5a-5f**.
Many of the volatile members of the acyclic esters 1a-h have very high repellency in the short-term assay. The formate 1a, the ester with the lowest molecular weight of any tested, was a significantly worse repellent in the longer assay than in the shorter study, though it was still moderately successful. In contrast, the isobutyrate 1b, which was statistically no different from 1a in the short term, provided no repellency in the long-term assay, despite its greater molecular weight (and likely lower volatility) than 1a, suggesting that 1a is inherently more potent than 1b. Citronellyl difluoroacetate (1f) was particularly repellent in the short-term assay, and provided very good repellency in the long-term study, suggesting that this compound is potentially a potent repellent with an acceptable vapor pressure. Previous work using monoterpenoid alcohols esterified with halogenated carboxylic acids suggest that these compounds are substantially more toxic to insects by fumigation than the structurally similar compounds using non-halogenated analogs,\textsuperscript{19, 27} a trend which may also affect the potency of these compounds as repellents.

Several compounds from this study, including 1g, were observed to have greater repellency in the long-term assay than in the short-term assay, a counterintuitive result. Although we are continuing to explore these unexpected results, there are several plausible explanations beyond a statistical type I error. There are several biological latent variables that may be responsible for this observation. For example, it is possible the biological variation between different cohorts of mosquitoes increases or reduces the susceptibility to a specific set of repellents, and that those tested in the long-term assay were more sensitive to the compound. Alternatively, turbulence within the testing chambers during the set up may disrupt the concentration of the repellent within the chamber, confounding the results in one of the assays. There is some evidence that other repellents, such as DEET, can act as olfactory agonists or
antagonists, depending on the concentration of the repellent,\textsuperscript{28} and it is therefore plausible that this may also contribute to the observed results.

The alicyclic esters series \textbf{2a-d} nicely captures a trend in repellency in the order expected from the molecular weight of the repellents, with the lightest ester (\textbf{2a}) being the most repellent. However, in the long-term assays, the cyclobutanecarboxylate \textbf{2b} drops substantially while \textbf{2a} maintains most of its repellent ability, suggesting that \textbf{2a} is more potent than \textbf{2b}. Modifying the cyclobutyl ring by adding substituents (\textbf{3a-d}) did not improve the efficacy of these compounds as repellents, and the lackluster performance of the fluorinated derivative \textbf{3b}, compared to \textbf{2a}, suggests that the naive substitution of hydrogen by fluorine does not necessarily improve the potency of a repellent.

As a class, the aromatic esters \textbf{4a-f} were not an improvement over the previous series of compounds. The benzoate ester \textbf{4a} was a particularly feeble repellent, with almost no repellency in the short-term study. While furoates and thiophenecarboxylates \textbf{4b-e} outperformed \textbf{4a} in the short-term assay, none of these compounds were particularly promising. Interestingly, there was no significant difference in repellency between the isomeric pairs \textbf{4b} and \textbf{4c}, or \textbf{4d} and \textbf{4e}, which used 2- and 3-substituted heteroarylcarboxylates. This result suggests that in these compounds, the aromatic ring is not substantially participating in the binding of these repellents to an odorant binding protein; it is also feasible that they do not fit into the receptor site of a putative odorant receptor. It is possible that some of the reduced repellency within this series of compounds is also due to the higher molecular weight compared to other series, with volatilities likely near that of \textbf{2d}, another poor repellent.

One of the best spatial repellents screened in this study was 2-hydroxyethyl citronellate, \textbf{5f}, which was highly repellent in both the short- and long-term assays. This result was somewhat
unexpected, as the presence of an unesterified hydroxyl group in the repellent was expected to significantly reduce volatility—\(5f\) is, in fact, an excellent contact repellent (data not shown). However, because the oxetanes \(5d\) and \(5e\) lack a hydrogen bond donor and are considerably less repellent than \(5f\), it is possible that the hydrogen bond donating ability of \(5f\) is important for repellency, particularly given that many repellent monoterpenoids are alcohols. The esters \(5b\) and \(5c\) were both good repellents in the short-term assay; however, \(5b\) provided almost no repellent effect after evaporating for five hours, in contrast to the closely related \(5c\). Given that these two esters differ in a single degree of unsaturation, it is possible that the terminal alkyne in \(5c\) plays an important role in the repellency of this compound.

**Conclusion**

To determine the effects of small structural changes in monoterpenoid-derived repellents, we have synthesized 28 citronellyl and citronellate esters and assayed their spatial repellency against *Culex pipiens* in both short- and long-term repellency assays. The efficacy of these compounds varied substantially, with several of the more promising repellents, such as 2-hydroxyethyl citronellate, providing substantial repellency even after 7.5 hours when tested in a static air chamber. By comparing esters of similar volatility, it is possible to make assumptions about the effects of the esterifying group on the innate potency of the repellent molecule, which may allow for further optimization of future repellents. We hope that this rational approach in assessing spatial repellency of citronellol-inspired esters proves useful in the future optimization of other monoterpenoid repellents.

**Acknowledgements**

This project was funded in part by the Deployed War-Fighter Protection Program project W911QY-17-1-0001. We would also like to thank the Iowa Agricultural Experimental Station for partial funding of this project.
References


11. Rice, P. J.; Coats, J. R., Insecticalid properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 1994, 87, 1172-1179.


Appendix

**Mean and variance of mosquito repellency assays**

Each individual trial $Y$ with $n$ total mosquitos is a binomial random variable $Y \sim \text{Bin}(n, p)$, where $p$ is the probability of finding a mosquito on a specified side of the repellency chamber; that is, the probability of a mosquito being repelled or not repelled. The traditional method of determining spatial mosquito repellency in a test chamber uses the formula

$$\%_{r} = \frac{100\%}{n}(Y_u - Y_t),$$

where $Y_u$ and $Y_t$ correspond to the binomial responses of the treated and untreated sides of the repellency chamber; however, while this method is useful for obtaining average percentage repellencies, using several tries to obtain estimates of $\%_{r}$, and then trying to obtain confidence intervals from these values should not be attempted. We first note that, after removing a constant factor of 100%,

$$\%_{0r} = \frac{(Y_u - Y_t)}{n}$$

$$= \frac{(n - Y_t) - Y_t}{n}$$
\[ = \left(1 - \frac{2Y_t}{n}\right), \]

and then

\[
E[\%_r] = E\left[\left(1 - \frac{2Y_t}{n}\right)\right] = \left(1 - E\left[\frac{2Y_t}{n}\right]\right) = (1 - 2p),
\]

and

\[
Var[\%_r] = Var\left[\left(1 - \frac{2Y_t}{n}\right)\right] = Var\left[\frac{2}{n} \cdot Y_t\right] = \left(\frac{2}{n}\right)^2 Var(Y_t) = \frac{4p(1-p)}{n}.
\]

We note that because \( p \in [0,1] \), we must have \( E[\%_r] \in [-1,1] \), and we therefore can immediately conclude that \( \%_r \) is neither binomially distributed nor normally distributed, and therefore normal approximations to confidence intervals for repellency data are likely not appropriate, and are liable to produce intervals extending beyond the interval \([-1,1]\).

**Overdispersion in repeated binomial sampling**

Several factors can cause \( p_t \) (and by symmetry, \( p_u \)) to vary between trials, including slight variations in the experimental set up (e.g., turbulence in the testing chamber, non-ideal diffusion of repellent, variation in the quantity of repellent on a treated paper, variations in the lighting and temperature of the assay) and biological aspects, such as the “litter-effect”. Under this varying probability \( p_t \), in each trial, we have the conditional probability \( Y_t|p_t \sim \)
Bin\((n, p_i)\), where \(i\) indicates the replicate. While the true distribution of \(p_i\) is not known, let \(E[p_i] = p\) and \(\text{Var}[p_i] = \sigma_p^2\). Then \(E[Y] = E_p[E_Y[Y_i|p_i]] = E_p[np_i] = np\), and

\[
\text{Var}(Y) = E_p[\text{Var}_Y[Y_i|p_i]] + \text{Var}_p[E_Y[Y_i|p_i]] \\
= E_p[np_i(1 - p_i)] + \text{Var}_p[np_i] \\
= n(E_p[p_i] - E_p[p_i^2]) + n^2\sigma_p^2 \\
= n \left( p - \text{Var}_p[p_i] - (E_p[p_i])^2 \right) + n^2\sigma_p^2 \\
= np(1 - p) + n(n - 1)\sigma_p^2,
\]

where \(\forall n \in \mathbb{Z}^+\), \(n(n - 1)\sigma_p^2 > 0\) is the overdispersion term.

The beta-binomial distribution is often used to account for overdispersion in binomial models\(^6\)-\(^8\) and by using the vector generalize linear and additive models (VGAM) package for R\(^9\), beta-binomial models can be fit to untransformed repellency data, and appropriate confidence intervals can be obtained.

**References**


CHAPTER 4. MONOTERPENOID CARBONATE ESTERS AS MOSQUITO REPELLENTS

James S. Klimavicz,\textsuperscript{1} Caleb L. Corona,\textsuperscript{1} and Joel R. Coats\textsuperscript{1}

\textsuperscript{1}Department of Entomology, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to Pest Management Science.

Abstract

Background

The most successful insect repellents commercially available for use, such as DEET, picaridin, and \( p \)-menthane-3,8-diol, are contact repellents, requiring that insects come into contact with a treated surface before they are repelled. In contrast to contact repellents, spatial repellents do not require physical contact with an arthropod, and could serve useful in preventing insects from entering enclosed areas. Although there has been a push to develop spatial repellents against mosquitoes, this area of research remains underexplored. As several spatially-repellent carboxylate esters of monoterpenoids providing long-term repellency have been recently developed, we sought to explore monoterpenoid carbonate esters as an alternative class of repellent molecules.

Results

A series of citronellyl carbonates were readily synthesized from chloroformates and citronellol, with the most effective spatial repellent, citronellyl ethyl carbonate, repelling approximately 90\% of mosquitoes in a short-term repellency assay extending out to 2.5 hours. Given the success of citronellyl ethyl carbonate, several other monoterpenoids were used to

\* JSK designed, synthesized, purified, and characterized all compounds for testing and wrote the manuscript, CLC performed the bioassay testing, and JRC assisted in the revision of the manuscript.
produce ethyl carbonates, most of which provided spatial repellency at or above 90% over the course of the bioassay.

**Conclusion**

These volatile monoterpenoid carbonate esters constitute a new class of spatial repellents that are similar to, but chemically distinct from, monoterpenoid carboxylate esters that have also been shown to provide good spatial repellency. As many of these carbonates have shown excellent repellency in the short-term bioassay, we hope to further explore these compounds as long-term spatial repellents, while expanding the number of parent monoterpenoids used to synthesized these compounds.

**Keywords**: repellents, mosquito, terpenoids, carbonates, biorational

**Introduction**

While significant gains have been made against malaria over the last twenty years, mosquitoes remain a threat to global health. Malaria, lymphatic filariasis, dengue, yellow fever, chikungunya, West Nile fever, and other mosquito-vectored diseases are responsible for substantial human morbidity and mortality. While annual global malaria deaths have fallen by more than 50% since 2000, nearly four billion people remain at risk of contracting arboviruses. While the use of insecticides remains an important component of global vector control strategy, the development of insecticide resistance in many populations of mosquitoes jeopardizes the advances made against these diseases. The use of insect repellents is an important part of integrated pest management strategies, and is complementary to pesticide use. In particular, spatial repellents are of interest for the exclusion of insects from entering spaces occupied by human hosts, and are complementary to other vector control methods like insecticide-treated mosquito nets and insecticide residual spraying.
Amongst the best-known arthropod repellents are \( N,N \)-diethyl-\( m \)-toluamide (DEET), picaridin, IR3535, \( p \)-menthane-3,8-diol (PMD).\(^{11}\) However, while these compounds provide protection from mosquito bites, they only do so through direct contact with the insect, as opposed to deterring insects from entering a volume of space.\(^{9-10}\) In contrast, plant essential oils have long been used as insecticides and insect repellents,\(^{12}\) and their complex compositions frequently contain volatile compounds that potentially lend themselves well for use as spatial repellents. These oils are frequently composed of mono- and sesquiterpenoid and phenylpropanoid hydrocarbons, alcohols, aldehydes, ketones, and esters, many of which are repellent or insecticidal.\(^{13}\) While readily available, these compounds are often not ideal for use as insect repellents due to their strong scents, high volatility and corresponding short duration of protection, and concerns regarding skin sensitivity.\(^{14-16}\)

Plant terpenoids are attractive starting points for the development of novel insect repellents as minor changes in structure can trigger significant changes in the potency of the compound, though it is normally not obvious how these changes will affect efficacy. A common strategy is to modify one or more functional groups in the parent terpenoid while leaving the bulk of the molecule intact. Methyl ethers of some monoterpenoids, such as the naturally-occurring methyl ethers of thymol and carvacrol, are less potent as repellents than the parent monoterpenoids.\(^{17-18}\) Terpenoid alcohols have been esterified to make repellent or insecticidal compounds; in many cases, these esters had improved physical properties, such as lower volatility and improved long-term spatial repellency, while others were better fumigants or contact insecticides.\(^{19-22}\) Monoterpenoid esters using glycine and \( \gamma \)-aminobutyric acid (GABA) residues have also been developed;\(^{23}\) these amino acids were chosen both because of their role as neurotransmitters and because many monoterpenoids have been found to be allosteric
modulators of insect GABA receptors.\textsuperscript{24} Although these amino esters had higher minimum effective doses for mosquito repellency than the parent monoterpenoids, they also slowly hydrolyse to release free monoterpenoids, which may provide for the extended release of repellent monoterpenoids.

Callicarpenal, a sesquiterpenoid found in \textit{Callicarpa spp.} (Verbenaceae), has previously been shown to be repellent to mosquitoes,\textsuperscript{25} and an epoxide derivative of this natural product was significantly more toxic to mosquitoes than the parent compound.\textsuperscript{26} Likewise, many synthetic derivatives of the related terpenoids valencene and nootkatone were more repellent than nootkatone itself against the Formosan termite \textit{Coptotermes formosanus} (Isoptera: Rhinotermitidae).\textsuperscript{27} Lactams derived from the natural monoterpenoid nepetalactone, a repellent component of catnip, \textit{Nepeta cataria} (Lamiaceae), essential oil,\textsuperscript{28} have also been synthesized as effective mosquito feeding deterrents.\textsuperscript{29}

Despite the push toward a greater exploration of the chemical space around spatially repellent molecules, surprisingly little work has been done to investigate carbonate esters as potential repellents. 4-Cycloocten-1-yl methyl carbonate, an artificial fragrance ingredient, has been patented as a repellent for some dipterans, including mosquitoes, house flies, and horn flies.\textsuperscript{30} Asymmetric carbonates of 1,3-dihydroxyacetone were also synthesized under the premise that these compounds might slowly hydrolyze to release repellent compounds,\textsuperscript{31} including 2-ethyl-1,3-hexanediol (Rutgers 612), a repellent that has fallen out of favor due to possible endocrine disruption after repeated exposure.\textsuperscript{32-33} Menthol propylene glycol carbonate, an artificial flavoring compound derived from the monoterpenoid menthol, has also been found to be an effective mosquito repellent.\textsuperscript{34-35} Because many monoterpenoid esters are successful mosquito repellents and fumigant, an examination of monoterpenoid carbonates was warranted
to assess the utility of this functional group for future development of novel repellent compounds.

**Materials and Methods**

**General information**

The monoterpenoids used for synthesis were purchased from Sigma-Aldrich, Acros, or TCI, and pyridine and triethylamine were both purchased from TCI. The chloroformates were purchased from Acros, TCI, or Alfa Aesar. All compounds were used as received. All solvents were purchased from Fisher Scientific and used as received. All NMR spectra were obtained at the Iowa State University Chemical Instrumentation Facility using Varian MR 400 MHz and Avance III 600 MHz spectrometers. Chemical shifts are reported relative to the residual solvent peak (CDCl$_3$: 7.26 ppm for $^1$H and 77.16 ppm for $^{13}$C; DMSO-$d_6$: 2.50 for $^1$H and 39.52 ppm for $^{13}$C) in ppm.36

**Carbonate synthesis**

In a typical procedure, under an argon atmosphere, monoterpenoid (10 mmol) was dissolved in chloroform (50 mL) along with pyridine (0.949 g, 12 mmol), and the solution was cooled to 0 ºC. A chloroformate (11 mmol) was added dropwise over two minutes at 0 ºC with stirring, and the reaction was then permitted to warm to 22 ºC, and was stirred at this temperature for 18 hours. The chloroform was removed under vacuum, and the residue was dissolved in ethyl acetate (30 mL) and water (50 mL). The organic layer was isolated, and washed with 1 M hydrochloric acid (20 mL), 1 M sodium hydroxide (20 mL), and brine (20 mL), followed by drying over anhydrous magnesium sulfate. Carbonate esters were all purified using column chromatography using 1:4 methyl t-butyl ether:hexane as eluent unless otherwise noted.

**Citronellyl ethyl carbonate (1a)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.08 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.13 – 4.03 (m, 4H), 2.08 – 1.87 (m, 2H), 1.78 – 1.68 (m, 1H), 1.68
(s, 1H), 1.59 (d, $J = 1.3$ Hz, 3H), 1.59 – 1.54 (m, 1H), 1.46 (dtd, $J = 13.5, 7.5, 6.0$ Hz, 1H), 1.39 – 1.27 (m, 1H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.18 (ddd, $J = 13.5, 9.3, 7.8, 6.0$ Hz, 1H), 0.91 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.4, 131.5, 124.7, 66.5, 63.9, 37.1, 35.7, 29.4, 25.8, 25.5, 19.4, 17.8, 14.4.

**Citronellyl methyl carbonate (1b)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.10 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.27 – 4.12 (m, 2H), 3.80 (s, 3H), 2.10 – 1.90 (m, 2H), 1.75 (ddd, $J = 13.4, 7.3, 5.1$ Hz, 1H), 1.70 (q, $J = 1.3$ Hz, 3H), 1.62 (d, $J = 1.3$ Hz, 3H), 1.61 – 1.55 (m, 1H), 1.49 (ddd, $J = 13.3, 8.0, 7.0, 6.0$ Hz, 1H), 1.37 (ddd, $J = 13.4, 9.5, 6.5, 5.4$ Hz, 1H), 1.20 (ddd, $J = 13.6, 9.4, 7.7, 6.0$ Hz, 1H), 0.94 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 156.0, 131.5, 124.6, 66.8, 54.8, 37.1, 35.6, 29.4, 25.8, 25.5, 19.4, 17.8.

**Citronellyl isopropyl carbonate (1c)** Isopropyl chloroformate (2 M in toluene) was used instead of neat isopropyl chloroformate. Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.08 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.87 (hept, $J = 6.3$ Hz, 1H), 4.23 – 4.07 (m, 2H), 2.08 – 1.87 (m, 2H), 1.77 – 1.68 (m, 1H), 1.68 (s, 1H), 1.59 (d, $J = 1.3$ Hz, 3H), 1.59 – 1.53 (m, 1H), 1.47 (ddd, $J = 13.4, 8.0, 7.2, 6.0$ Hz, 1H), 1.35 (ddd, $J = 13.6, 7.1, 5.3, 4.1$ Hz, 1H), 1.29 (d, $J = 6.3$ Hz, 7H), 1.18 (ddd, $J = 13.6, 9.4, 7.8, 6.0$ Hz, 1H), 0.91 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.0, 131.5, 124.7, 71.8, 66.3, 37.1, 35.7, 29.4, 25.8, 25.5, 21.9, 19.5, 17.8.

**Citronellyl isobutyl carbonate (1d)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.08 (thept, $J = 7.1, 1.4$ Hz, 1H), 4.24 – 4.09 (m, 2H), 3.91 (d, $J = 6.7$ Hz, 3H), 2.06 – 1.90 (m, 4H), 1.73 (ddd, $J = 13.4, 7.3, 5.1$ Hz, 1H), 1.67 (d, $J = 1.3$ Hz, 3H), 1.59 (d, $J = 1.4$ Hz, 3H), 1.59 – 1.54 (m, 1H), 1.54 – 1.41 (m, 1H), 1.35 (ddd, $J = 13.3, 9.5, 6.4, 5.3$ Hz, 1H), 1.18 (ddd, $J = 13.6, 9.4, 7.7, 6.0$ Hz, 1H), 0.95 (d, $J = 6.7$ Hz, 6H), 0.91 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101
MHz, CDCl$_3$) δ 155.6, 131.5, 124.6, 74.1, 66.6, 37.1, 35.6, 29.3, 27.9, 25.9, 25.5, 19.5, 19.1, 17.8.

Citronellyl t-butyl carbonate (1e) Citronellol (1.56 g, 10 mmol) and 4-(dimethylamino)pyridine (224 mg, 2 mmol) were dissolved in dichloromethane (50 mL). Di-tert-butyl dicarbonate (2.40 g, 11 mmol) was added in one portion, and the reaction was stirred at 22 ºC for 12 hours. The solvent was removed under vacuum, and the reaction was worked up as in the general procedure. The title compound was purified as above to yield a colorless oil (1.98 g, 77%). $^1$H NMR (400 MHz, CDCl$_3$) δ 5.08 (thept, $J = 7.1$, 1.4 Hz, 1H), 4.24 – 4.09 (m, 2H), 2.07 – 1.91 (m, 4H), 1.77 – 1.67 (m, 1H), 1.69 (d, $J = 1.3$ Hz, 3H), 1.61 (d, $J = 1.4$ Hz, 3H), 1.58 – 1.53 (m, 1H), 1.52 (s, 9H), 1.51 – 1.43 (m, 1H), 1.37 (dddd, $J = 13.3$, 9.6, 6.5, 5.3 Hz, 1H), 1.20 (dddd, $J = 13.4$, 9.5, 7.7, 6.0 Hz, 1H), 0.91 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.6, 131.5, 124.7, 81.9, 66.5, 37.1, 35.6, 29.4, 27.9, 25.9, 25.5, 19.5, 17.8.

Citronellyl phenyl carbonate (1f) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.43 – 7.34 (m, 2H), 7.26 – 7.21 (m, 1H), 7.20 – 7.16 (m, 2H), 5.10 (thept, $J = 7.1$, 1.4 Hz, 1H), 4.37 – 4.22 (m, 2H), 2.09 – 1.93 (m, 2H), 1.81 (dtd, $J = 13.3$, 7.3, 4.9 Hz, 1H), 1.72 – 1.63 (m, 1H), 1.69 (q, $J = 1.3$ Hz, 3H), 1.62 (d, $J = 1.4$ Hz, 3H), 1.60 – 1.51 (m, 1H), 1.39 (dddd, $J = 13.4$, 9.4, 6.5, 5.3 Hz, 1H), 1.22 (dddd, $J = 13.6$, 9.4, 7.7, 6.1 Hz, 1H), 0.96 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 153.9, 151.3, 131.6, 129.6, 126.1, 124.6, 121.2, 67.5, 37.1, 35.6, 29.4, 25.9, 25.5, 19.5, 17.8.

Ethyl thymyl carbonate (2a) Colorless oil (1.78 g, 80%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 (d, $J = 7.9$ Hz, 1H), 7.04 (dd, $J = 8.3$, 1.1 Hz, 1H), 6.91 (d, $J = 1.0$ Hz, 1H), 4.32 (q, $J = 7.1$ Hz, 2H), 3.08 (hept, $J = 6.9$ Hz, 1H), 2.32 (s, 3H), 1.39 (t, $J = 7.1$ Hz, 3H), 1.21 (d, $J = 6.9$ Hz,
6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 154.1, 148.4, 137.2, 136.8, 127.5, 126.7, 122.5, 64.9, 27.1, 23.2, 21.0, 14.4.

**Isobutyl thymyl carbonate** (2d) Triethylamine (1.21 g, 12 mmol) used instead of pyridine. Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.19 (d, $J = 7.9$ Hz, 1H), 7.04 (dd, $J = 7.7$, 1.8 Hz, 1H), 6.91 (d, $J = 2.1$ Hz, 1H), 4.05 (d, $J = 6.7$ Hz, 1H), 3.08 (hept, $J = 6.9$ Hz, 1H), 2.32 (s, 1H), 2.06 (thept, $J = 6.9$, 6.7 Hz, 1H), 1.21 (d, $J = 6.9$ Hz, 3H), 1.01 (d, $J = 6.7$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 194.3, 154.3, 148.4, 137.2, 136.8, 127.5, 126.7, 122.5, 74.8, 28.0, 27.1, 23.2, 21.0, 19.0.

**Ethyl isopulegyl carbonate** (3a) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 4.773 (s, 1H), 4.769 (s, 1H), 4.62 (td, $J = 10.9$, 4.4 Hz, 1H), 4.20 – 4.08 (m, 2H), 2.19 – 2.02 (m, 2H), 1.77 – 1.65 (m, 2H) 1.69 (s, 1H), 1.54 (dtdt, $J = 16.4$, 10.1, 6.8, 3.2 Hz, 1H), 1.37 (qd, $J = 14.5$, 13.8, 4.1 Hz, 1H), 1.27 (t, $J = 7.2$ Hz, 3H), 1.09 (td, $J = 12.2$, 11.0 Hz, 1H), 0.94 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 154.8, 145.8, 112.0, 77.4, 63.6, 50.5, 40.3, 34.0, 31.4, 30.4, 22.0, 19.6, 14.2.

**Ethyl geranyl carbonate** (4a) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.37 (th, $J = 7.1$, 1.3 Hz, 1H), 5.07 (thept, $J = 6.8$, 1.5 Hz, 1H), 4.64 (dd, $J = 7.2$, 0.9 Hz, 2H), 4.19 (q, $J = 7.1$ Hz, 2H), 2.17 – 1.97 (m, 4H), 1.71 (d, $J = 1.4$ Hz, 3H), 1.67 (d, $J = 1.4$ Hz, 3H), 1.59 (d, $J = 1.4$ Hz, 3H), 1.30 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.4, 143.2, 132.0, 123.8, 117.9, 64.6, 64.0, 39.6, 26.4, 25.8, 17.8, 16.6, 14.4.

**Ethyl menthyl carbonate** (5a) Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 4.50 (td, $J = 10.9$, 4.5 Hz, 1H), 4.18 (qd, $J = 7.1$, 2.3 Hz, 2H), 2.07 (dtd, $J = 12.0$, 4.1, 1.8 Hz, 1H), 1.97 (heptd, $J = 7.0$, 2.7 Hz, 1H), 1.73 – 1.62 (m, 2H), 1.48 (dddt, $J = 15.2$, 8.6, 6.5, 3.3 Hz, 0H), 1.40 (ddt, $J = 12.4$, 10.8, 3.1 Hz, 1H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.12 – 0.98 (m, 2H), 0.90 (t, $J =
7.0 Hz, 6H), 0.78 (d, J = 7.0 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.0, 78.2, 63.8, 47.1, 40.9, 34.2, 31.5, 26.1, 23.4, 22.1, 20.9, 16.3, 14.4.

**Ethyl perillyl carbonate (6a)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.80 (tt, J = 2.9, 1.3 Hz, 1H), 4.75 – 4.67 (m, 2H), 4.55 – 4.45 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 2.21 – 2.06 (m, 4H), 2.02 – 1.90 (m, 1H), 1.84 (ddq, J = 10.6, 4.1, 2.2 Hz, 1H), 1.73 (d, J = 1.2 Hz, 3H), 1.55 – 1.40 (m, 1H), 1.30 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.4, 149.7, 132.3, 126.8, 108.9, 71.9, 64.1, 40.8, 30.6, 27.4, 26.4, 20.9, 14.4.

**Bornyl ethyl carbonate (7a)** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 4.80 (ddd, J = 9.9, 3.5, 2.1 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 2.37 (dddd, J = 13.6, 9.9, 4.7, 3.3 Hz, 1H), 2.01 – 1.89 (m, 1H), 1.82 – 1.70 (m, 1H), 1.68 (t, J = 4.5 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H), 1.30 – 1.21 (m, 1H), 1.09 (dd, J = 13.8, 3.5 Hz, 1H), 0.90 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.7, 83.8, 63.9, 49.0, 48.1, 44.9, 36.6, 28.1, 27.0, 19.9, 19.0, 14.5, 13.6.

**Mosquito repellency testing**

The static air chamber used for the repellency assay has been described previously. For each assay run, a 90-mm Whatman No. 1 filter paper was treated with 1 mL acetone solution containing 0.5% w/v of a carbonate ester. The filter paper was supported upon several pins during the addition of the acetone solution to diminish wicking of the solution away from the filter paper, and the solution was added over approximately thirty seconds to allow acetone to evaporate during the addition to prevent dripping. After the addition of the solution was complete, remaining acetone was permitted to evaporate for 10 minutes before testing.

A 600-mm-long clear glass cylinder with an inner diameter of 85 mm and a single hole (20 mm diameter) halfway along the side of the cylinder was used for the repellency chamber. One end of the chamber was capped with a glass petri dish (inner diameter 90 mm) containing an untreated 90-mm Whatman No. 1 filter paper, while the other end was likewise covered with a
similar petri dish containing the treated filter paper. Immediately after capping both ends of the chamber, 20 non-blood-fed female *Culex pipiens* mosquitoes were anesthetized with carbon dioxide, and placed into the repellency chamber through the hole in the side of the cylinder via a funnel. The hole was sealed, and the number of mosquitoes on the treated and untreated halves were recorded at 15, 30, 60, 90, 120, and 150 minutes after introduction of the mosquitoes to the chamber.

**Data analysis**

All statistical analysis was performed in R. To determine repellency of a given compound, the number of repelled mosquitoes and the total number of mosquitoes the chamber for each trial at a given time point were fit to a beta-binomial distribution to account for overdispersion in the estimated repellency (see the Appendix of Chapter 3 for details). Estimates of the proportion of mosquitoes repelled in the chamber was then transformed to percentage repellency, which is expressed as

\[
\%_{r} = 100\% \cdot \frac{n_{t} - n_{u}}{n_{t} + n_{u}}.
\]

This formula ensures that percentage repellency is bound between -100% (completely attracting) and 100% (completely repelling), and percentage repellency is shown with error bars representing 50% confidence intervals determined from a beta-binomial fit to the data, which accommodates overdispersion present in the data. Statistical significance was determined by nonoverlap of these confidence intervals.

**Results and Discussion**

We first elected to make a series of citronellyl carbonates because amongst a series of isovalerate esters that we have previously tested as short- and long-term mosquito repellents, citronellyl isovalerate provided a moderate response, allowing us to make distinctions between
different levels of efficacy. Using commercially available ethyl chloroformate, we synthesized citronellyl ethyl carbonate (1a) as our first carbonate, since this choice of alkyl group produced a compound with a molecular weight most similar to sesquiterpenoid alcohols, which has previously been successful as a strategy in developing long-lasting spatial mosquito repellents. Using other chloroformates and citronellol, the corresponding methyl, isopropyl, isobutyl, and phenyl carbonates (1b, 1c, 1d, and 1f, respectively) were also synthesized. We also produced citronellyl tert-butyl carbonate (1e) using di-tert-butyl dicarbonate. The short-term repellency trends of these compounds are shown in Figure 4.1. Of the compounds in this series, the somewhat bulky 1f produced the weakest repellent effect, and was statistically significantly poorer than many of the other carbonates, all of which had lower molecular weight than 1f. Repellent 1a was numerically superior out of all the citronellyl carbonates, while the methyl, isopropyl, and isobutyl derivatives were numerically, but not statistically, less repellent than 1a.

Figure 4.1. Short-term repellency of citronellyl carbonates against *Culex pipiens*. 

![Figure 4.1. Short-term repellency of citronellyl carbonates against *Culex pipiens*.](image-url)
Given the success of 1a, we then elected to synthesize other ethyl monoterpenoid carbonates to explore trends in short-term repellency; these results are shown in Figure 4.2. A selection of structurally diverse monoterpenoids was chosen for synthesis and testing and comparison to 1a. The thymyl, isopulegyl, geranyl, menthyl, and perillyl ethyl carbonates (compounds 2a, 2b, 2c, 2d, and 2e, respectively) were readily synthesized from their corresponding monoterpenoid alcohols, and showed remarkably good short-term repellency, with all of 1a and 2a-e repelling 90% or more of the C. pipiens after the 90-minute time point. Bornyl ethyl carbonate (7a), again easily synthesized from borneol, was the one exception to the high repellency of these ethyl carbonates, and only modest repellency was observed, significantly lower than that seen for the other monoterpenoid ethyl carbonates. We also
attempted to synthesize the ethyl carbonates of linalool and α-terpineol; however, the reaction was unsuccessful with these sterically-hindered tertiary monoterpenoids.

Isobutyl thymyl carbonate (2d) was then produced to determine if other monoterpenoid isobutyl carbonates are similar in repellency to 1d. The results of this examination are shown in Figure 4.3. Ethyl thymyl carbonate was an excellent short-term repellent, and achieves high repellency in the static air chamber much more quickly than does the isobutyl analog, as 2d more closely follows the pattern of the corresponding citronellol derivative 1d. This slower onset of high repellency may be a result of the higher molecular weight, since the final estimated repellency was approximately the same for both 2a and 2d.
Conclusion

Volatile monoterpenoid carbonates represent a new class of spatial insect repellents, and many of these compounds are excellent spatial repellents against *Culex pipiens*. In particular, most of the monoterpenoid ethyl carbonates screened in this study repelled 90% or more of the mosquitoes in our bioassay. We expect that continued exploration of these new molecules and their capacity to repel mosquitoes in long-term assays will yield exciting results.

Notes and Acknowledgements

The appendix contains several carbonate esters that have been synthesized, but have not been assayed for repellency by the time of submission of this dissertation. Additionally, long-term repellency testing has yet to be performed on these compounds. This project was funded in part by the Deployed War-Fighter Protection Program project W911QY-17-1-0001 and the Iowa Agricultural Experimental Station.

References


15. de Groot, A. C.; Frosch, P. J., Adverse reactions to fragrances. *Contact Dermatitis* **1997**, 36, 57-86.


22. Rice, P. J.; Coats, J. R., Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1994**, *87*, 1172-1179.


**Appendix**

The following carbonates were synthesized but not tested against mosquitoes in time for this dissertation.

![Allyl citronellyl carbonate](image)

Allyl citronellyl carbonate Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$)

$^\delta$ 5.94 (dddt, $J = 17.2, 10.4, 5.8$ Hz, 1H), 5.36 (dq, $J = 17.2, 1.5$ Hz, 1H), 5.26 (dq, $J = 10.4, 1.3$ Hz, 1H), 5.08 (tdq, $J = 7.1, 2.8, 1.4$ Hz, 1H), 4.62 (dt, $J = 5.8, 1.4$ Hz, 2H), 4.25 – 4.10 (m, 2H), 2.08 – 1.88 (m, 2H), 1.77 – 1.68 (m, 1H), 1.68 (q, $J = 1.3$ Hz, 3H), 1.60 (d, $J = 1.3$ Hz, 3H), 1.62
2-Chloroethyl citronellyl carbonate
Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.08 (thept, $J = 7.1$, 1.4 Hz, 1H), 4.37 (dd, $J = 6.3$, 5.4 Hz, 2H), 4.24 - 4.14 (m, 2H), 3.70 (dd, $J = 6.3$, 5.4 Hz, 2H), 2.04 - 1.88 (m, 1H), 1.73 (dtd, $J = 13.6$, 7.4, 5.2 Hz, 1H), 1.68 (d, $J = 1.4$ Hz, 3H), 1.60 (d, $J = 1.3$ Hz, 3H), 1.59 - 1.53 (m, 1H), 1.48 (dddd, $J = 13.3$, 8.0, 7.1, 6.0 Hz, 1H), 1.35 (dddd, $J = 13.4$, 9.5, 6.4, 5.3 Hz, 1H), 1.19 (dddd, $J = 13.6$, 9.3, 7.7, 6.0 Hz, 1H), 0.92 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.0, 131.6, 124.6, 67.2, 41.3, 37.1, 35.6, 29.3, 25.8, 25.5, 19.4, 17.8.

Ethyl verb-enyl carbonate
Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.39 (dhept, $J = 3.2$, 1.6 Hz, 1H), 5.36 (tq, $J = 3.4$, 1.7 Hz, 1H), 4.18 (q, $J = 7.1$ Hz, 2H), 2.50 (dd, $J = 9.1$, 6.3, 5.2 Hz, 1H), 2.41 (tdd, $J = 5.9$, 3.3, 1.9 Hz, 1H), 2.00 (td, $J = 5.5$, 1.3 Hz, 1H), 1.74 (t, $J = 1.7$ Hz, 3H), 1.40 (d, $J = 9.1$ Hz, 1H), 1.34 (s, 3H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.02 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.2, 150.4, 115.3, 79.2, 63.8, 47.7, 45.6, 39.8, 35.7, 26.7, 22.8, 22.7, 14.5.

Ethyl myrtenyl carbonate
Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.61 (tp, $J = 2.9$, 1.4 Hz, 1H), 4.49 (ABqq, $J_{AB} = 12.3$ Hz, $J = 4.8$ Hz, 2H), 4.18 (q, $J = 7.1$ Hz, 2H), 2.40 (dt, $J = 8.7$, 5.6 Hz, 1H), 2.28 (ABqtt, $J_{AB} = 18.1$ Hz, $J = 3.1$, 1.6 Hz, 2H), 2.17 (td, $J = 5.6$, 1.5 Hz, 1H), 2.09 (ttq, $J = 5.8$, 2.8, 1.2 Hz, 1H), 1.30 (t, $J = 7.1$ Hz, 4H), 1.28 (s, 3H), 1.18 (d, $J = 8.7$ Hz, 1H), 0.82 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.4, 142.6, 122.5, 70.5, 64.0, 43.5, 40.8, 38.2, 31.6, 31.4, 26.2, 21.1, 14.5.
**Ethyl carvacryl carbonate** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.14 (d, $J = 7.8$ Hz, 1H), 7.03 (dd, $J = 7.8$, 1.8 Hz, 1H), 6.96 (d, $J = 1.8$ Hz, 1H), 4.32 (q, $J = 7.1$ Hz, 2H), 2.88 (hept, $J = 6.9$ Hz, 1H), 2.20 (s, 3H), 1.40 (t, $J = 7.1$ Hz, 3H), 1.23 (d, $J = 6.9$ Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 153.7, 149.6, 148.3, 131.1, 127.2, 124.4, 119.6, 64.9, 33.7, 24.0, 15.7, 14.4.

**Cinnamyl ethyl carbonate** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.42 – 7.38 (m, 2H), 7.35 – 7.30 (m, 2H), 7.27 (tt, $J = 6.2$, 1.5 Hz, 1H), 6.69 (dt, $J = 15.9$, 1.4 Hz, 1H), 6.30 (dt, $J = 15.9$, 6.5 Hz, 1H), 4.79 (dd, $J = 6.4$, 1.3 Hz, 2H), 4.23 (q, $J = 7.1$ Hz, 2H), 1.33 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.2, 136.2, 134.8, 128.7, 127.6, 122.6, 68.3, 64.2, 14.4.

**Ethyl eugenyl carbonate** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.04 (d, $J = 8.0$ Hz, 1H), 6.79 (d, $J = 1.9$ Hz, 1H), 6.76 (dd, $J = 8.0$, 1.9 Hz, 1H), 5.95 (ddt, $J = 16.9$, 10.1, 6.7 Hz, 1H), 5.14 – 5.07 (m, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 3.84 (s, 3H), 3.38 (dt, $J = 6.9$, 1.6 Hz, 2H), 1.38 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 153.6, 151.1, 139.3, 138.5, 137.1, 122.3, 120.7, 116.4, 112.9, 65.0, 56.0, 40.2, 14.3.

**Methyl thymyl carbonate** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 (d, $J = 7.9$ Hz, 1H), 7.05 (dd, $J = 7.9$, 1.3 Hz, 0H), 6.91 (d, $J = 0.9$ Hz, 1H), 3.91 (s, 3H), 3.08 (hept, $J = 6.9$ Hz, 1H), 2.33 (s, 3H), 1.21 (d, $J = 6.9$ Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 154.6, 148.2, 137.0, 136.7, 127.4, 126.5, 122.2, 55.4, 26.9, 23.1, 20.8.

**Isopropyl thymyl carbonate** Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 (d, $J = 7.9$ Hz, 1H), 7.04 (dd, $J = 7.8$, 1.9 Hz, 1H), 6.91 (d, $J = 1.4$ Hz, 1H), 4.98 (hept, $J = 6.2$ Hz, 1H), 3.08 (hept, $J = 6.9$ Hz, 1H), 2.33 (s, 3H), 1.39 (d, $J = 6.3$ Hz, 6H), 1.21 (d,
$J = 6.9 \text{ Hz, 7H}). \ \text{^{13}C NMR (101 MHz, CDCl}_3 \ \delta 153.7, 148.3, 137.2, 136.8, 127.4, 126.6, 122.5, 73.0, 27.1, 23.2, 21.9, 21.0.$

<chemistry>
\begin{align*}
\text{Ethyl (3(5)-isopropyl-7-oxocyclohepta-1,3,5-trien-1-yl) carbonate}
\end{align*}
</chemistry>

From hinokitiol and ethyl chloroformate.

Light brown oil. $^1\text{H NMR (400 MHz, CDCl}_3 \ \delta 7.21 \text{ (s, 1H), 7.16 \text{ (d, } J = 10.6 \text{ Hz, 1H), 7.07 (broad s, 1H), 6.97 \text{ (d, } J = 9.1 \text{ Hz, 1H), 4.32 \text{ (q, } J = 7.1 \text{ Hz, 2H), 2.89 – 2.72 \text{ (m, } J = 6.9 \text{ Hz, 1H), 1.38 \text{ (t, } J = 7.1 \text{ Hz, 3H), 1.23 \text{ (d, } J = 6.8 \text{ Hz, 6H). Missing } ^{13}\text{C NMR signals and broadened tropolone } ^1\text{H NMR peaks possibly due to rapid intramolecular isomerization as described previously for tropolone acetates.}^1$

Reference

CHAPTER 5. FLUORESCENT HIGH-THROUGHPUT SCREENING OF MONOTERPENOIDS AND NEMATICIDES USING C. ELEGANS EGGS

James S. Klimavicz,¹* Claire Pouliot,¹ and Joel R. Coats¹

¹Department of Entomology, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to Pest Management Science.

Abstract

Background

Caenorhabditis elegans is frequently used as a model organism in toxicity screening and mode of action studies. Many C. elegans-based bioassays use techniques that preclude high-throughput screening. By using fluorescein diacetate (FDA) as a probe to determine viability of C. elegans after exposure to potential toxicants, a new fluorescence-based bioassay was developed to rapidly assay the toxicity of natural monoterpenoids and phenylpropanoids, several commercially-available analogs of cinnamaldehyde, and several commercial nematicides. C. elegans were reared using standard techniques, and synchronized eggs were placed into 96-well plates containing various levels of compounds of interest. Over a 24-hour period, the viable eggs, as well as the worms that hatched, hydrolyze FDA to produce fluorescein, which is then quantified using a plate reader.

Results

Of the 19 monoterpenoids and natural phenylpropanoids screened, only limonene (96 ppm), cinnamaldehyde (73 ppm), and geraniol (49 ppm) resulted in measurable EC₅₀ values below 128 ppm. Several cinnamaldehyde analogs were also tested, the best of which, 4-methoxycinnamaldehyde, had an estimated EC₅₀ value of 38.9 ppm. Abamectin and oxamyl, two

* JSK synthesized all synthetic compounds for testing, designed the nematicide screening bioassay, and wrote the manuscript, CLC performed most of the bioassay testing. JRC assisted in the revision of the manuscript.
nematicides available as agricultural nematicides, produced EC\textsubscript{50} values of 80.6 and 140 ppm, respectively. At low concentrations of many compounds, an increase in fluorescence was observed as compared to the control; this is suspected to be a result of increased esterase production by the \textit{C. elegans} in response to the presence of these compounds.

\textbf{Conclusion}

While many natural monoterpenoids and phenylpropanoids do not cause significant mortality below 128 ppm, this FDA-based fluorescent screening assay provides a rapid method of screening the toxicity of compounds without the need to assess individual nematodes. Moreover, this assay permits the determination of toxic doses, and compounds with the potential to induce increased metabolic activity at sublethal concentrations of toxicants.

\textbf{Keywords:} \textit{C. elegans}, high-throughput screening, fluorescence assay, nematicide

\textbf{Introduction}

\textit{Caenorhabditis elegans} is an invaluable model for pharmaceutical screening and toxicity testing in multicellular organisms,\textsuperscript{1-3} as it is an easily-reared, fast-growing multicellular species. \textit{C. elegans} has also been used as a model for parasitic nematode research and nematicide screening,\textsuperscript{4} and for the discovery of new anthelmintic compounds.\textsuperscript{5} Many \textit{C. elegans}-based bioassays use techniques that require the assessment of single worms to characterize mortality or behavioral changes, which requires either expensive worm-counting equipment and software,\textsuperscript{6} expensive microfluidic devices,\textsuperscript{7} or the manual observation of individual nematodes with the concomitant reduction in the throughput of screening.\textsuperscript{8-9} Transgenic \textit{C. elegans} expressing green fluorescent protein have also been used in high-throughput screening.\textsuperscript{10} Colorimetric assays allow for higher throughput, but frequently require either the use of an expensive water-soluble dye, or an additional dye-solubilization step. An ideal high-throughput assay would use readily-available, low-cost reagents, and would allow for rapid screening of compounds.
The use of tetrazolium salts, such as 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for colorimetric assays, forming highly-colored formazans through mitochondrial reduction.\textsuperscript{11-12} However, MTT is relatively expensive, and the formazan produced by the nematodes forms small crystals within the worms as the formazan is not soluble in water. Quantitation of the formazan therefore requires that the nematodes be pelleted by centrifugation, aspiration of the supernatant, and solubilization of the formazan by the addition of DMSO. MTT analogs that produce water-soluble formazans are also available, but these compounds are also expensive.

Fluorescein diacetate (FDA) is a non-fluorescent, cell-permeable compound that is hydrolyzed by esterases into the fluorescein, which fluoresces at physiological pH. FDA assays have been used previously to quantify the growth of yeast and bacteria and to assess viability of mouse embryos,\textsuperscript{13-15} while other fluorescein derivatives have been used for other organismal studies.\textsuperscript{16-17}

A previous investigation looked at the lethality of monoterpenoids and related compounds on \textit{C. elegans} and calculated \textit{LC}_{50} values for some of these compounds;\textsuperscript{18} however, the utility of this work is severely limited by ill-fitted dose-response curves and a lack of confidence intervals on the obtained \textit{LC}_{50} values.

**Materials and Methods**

**Nematode culture**

\textit{C. elegans} (Bristol N2 strain, Carolina Biological Supply) were reared according to known methods.\textsuperscript{19} Briefly, nematode growth medium (NGM) was prepared by the addition of sodium chloride (3 g), agar (17 g), and peptone (2.5 g) to distilled water (975 mL) in a 2 L conical flask, followed by sterilization by autoclaving. After cooling to 60 °C, 1 M calcium chloride (1 mL), 1 M magnesium sulfate (1 mL), 1 mg mL\textsuperscript{-1} cholesterol in ethanol (1 mL), and
1 M potassium phosphate buffer (25 mL, pH 6) were added, and the solution was mixed well. The warm NGM was poured into 10 cm petri plate, and allowed to cool. *E. coli* (OP50 strain, Carolina Biological Supply) was streaked onto a petri plate containing LB agar (prepared from 10 g tryptone, 5 g yeast extract, 5 g sodium chloride, and 15 g agar in 1 L water, with the pH adjusted to 7.5). A single colony of *E. coli* was then cultured in 100 mL of Luria broth—prepared from 10 g tryptone, 5 g yeast extract, and 5 g sodium chloride in 1 L water, with the pH adjusted to 7.0—in a 250 mL culture flask overnight at 35 ºC. NGM plates were seeded by pipetting 100 μL of *E. coli* culture onto the plate, and allowing the *E. coli* to grow for 16 hours at 35 ºC.

Nematode cultures were propagated through the chunking method, wherein a 1 cm² square of agar is removed from an old plate of nematodes and placed onto a new plate seeded with *E. coli*. After chunking, plates were incubated for 72 hours before harvesting nematodes for synchronization.

**Synchronization**

Synchronization of *C. elegans* was performed using a standard alkaline hypochlorite method. Nematodes were collected by rinsing plates containing gravid adults with distilled water and collecting the liquid in 15 mL centrifuge tubes. The worms were pelleted by centrifugation at 1000 × *g* for 1 min, and the supernatant was discarded. Distilled water was added to a volume of 3.5 mL, followed by the addition of 5 M sodium hydroxide (0.5 mL) and 6% sodium hypochlorite (1 mL Clorox® regular bleach). The tube was vortexed for 10 seconds every two minutes for a total of 10 minutes, and the eggs were then collected by centrifugation at 1500 × *g* for 1 min. The supernatant was discarded, and the eggs were washed with water once, and M9 buffer (prepared from 3 g anhydrous monopotassium phosphate, 6 g anhydrous disodium phosphate, and 1 mL 1 M magnesium sulfate in 1 L water, autoclaved for sterility, and pH-
adjusted to 7.0) twice, resuspending the eggs, pelleting by centrifugation at $1500 \times g$ for 1 min, and discarding the supernatant each time. The egg pellet is then resuspended in M9 buffer to a concentration of approximately 5,000 eggs mL$^{-1}$ for use in the bioassay, using a hemocytometer to estimate egg concentration.

**FDA assay**

Stock solutions of each potential nematicide were made by dissolving 25.6 mg of the compound of interest in a solution of 10% Triton-X100 in ethanol in a 1 mL volumetric flask to produce a 25600 ppm (200×) solution. M9 buffer with 0.1% Triton-X-100 and 0.9% ethanol (M9TE) was made by adding 10 mL of 10% Triton-X100 in ethanol to 990 mL of M9 buffer.

The fluorescence assays were performed in transparent, flat-bottomed 96-well culture plates (Corning® product number 3997). To set up the bioassay, 10 μL of the 200× stock solution was added to 990 μL of M9 buffer to produce a 2× solution of the potential nematicide. To each well of the first column of the 96-well plate was added 200 μL of 2× AI solution. To each well of columns 2–12 was added 100 μL of M9TE. Two-fold serial dilutions were then performed using an 8-channel pipet to transfer 100 μL from each well in the first column to the corresponding well in the second column, and mixing well. The process was repeated from the second column to the third, and then continuing down to the tenth column, after which 100 μL is discarded to ensure that each well in the microplate contains 100 μL of liquid, and columns 1–10 contain AI ranging from 256 ppm down to 0.5 ppm. To each well in columns 1–11 was added 100 μL of synchronized *C. elegans* eggs (approximate 500 eggs); to each well in column 12 was added 100 μL of M9 buffer, giving final AI concentrations of 128 ppm down to 0.25 ppm, with a negative control in column 11, and a blank in column 12. Plates were typically set up to either contain two replicates each of four different potential nematicides, or four replicates each of two nematicides.
The 96-well plate was covered with a lid and was sealed with Parafilm®, and incubated at 20 °C on a plate rocker for 4 hours. To 1.96 mL of M9 buffer was added 44 μL of FDA in DMSO (10 mg mL⁻¹) stock solution, and 20 μL of this solution was added to each well. The plate was then resealed with the lid and new Parafilm, and was incubated at 20 °C on a plate rocker for 20 hours. The formation of fluorescein was then quantified using a Gen5™ software with a Synergy HTX plate reader (Biotek®) in filter-based fluorescence mode (excitation: 20 nm bandwidth centered at 485 nm; emission: 20 nm bandwidth centered at 528 nm).

**Data analysis**

All data analysis was done in R, and dose-response curve-fitting and analysis were performed using the *drc* package. Results from plates in which the fluorescence values of the treated wells of a treatment was significantly lower than the mean blank well (Column 12) value were not used due to possible contamination; the mean blank value was then subtracted from each well. For each row of a plate, the fluorescence values of the treated wells were used to fit a four-parameter logistic regression data, and this curve was used to normalize the fluorescence values in the row by setting the low-concentration asymptote of the dose-response curve to 1. After normalization, the combined data from all replicates for a treatment was used to fit both a regression accounting for a hormesis-type effect using the CRS models, and a three- or four-parameter logistic regression. The models used were of the formula

\[
\varphi(x|b, c, d, e, f, \alpha) = c + \frac{d - c + f \exp[-x^{-a}]}{1 + \exp[b(\ln(x) - \ln(e))]},
\]

where \(\alpha\) and \(f\) were fixed at 0 in the logistic regressions, and \(c\) was fixed as well in the three-parameter logistic regression. In the CRS models, \(c\) was fixed at 0, and \(\alpha\) was set at 0.25, 0.5, or 1, as programmed in the *drc* package, or allowed to vary to permit a better fit, as derived in the
Appendix of this chapter. The best-fitting model was determined using the Akaike information criterion (AIC). The best-fitting model was then used for determination of EC_{50} values.

**Results and Discussion**

Our first step was to develop a fluorescent bioassay that would produce consistent results. Because FDA hydrolyzes slowly but measurably at room temperature around pH 7.0, it was important to ensure that a good signal-to-noise ratio could be obtained in the bioassay. Based on results from previous colorimetric assays using *C. elegans*, we first explored the effects of the number of nematode eggs in each well of a 96-well plate, as shown in Figure 5.1a. The measured fluorescence increases approximately linearly, as determined by a coefficient of 0.9848 in the log-log plot. At a nominal 2000 eggs per well, the fluorescence measurement was below this fit, suggesting that adding too many eggs diminishes the efficiency of the assay. Figure 5.1b shows the signal-to-noise (SNR) of these measurements, which shows that the SNR increases roughly linearly with the square of the number of eggs in each well, as expected from theory. As a compromise between SNR and the logistics of having large numbers of eggs in each well, we elected on using approximately 500 eggs per well.

Because the rate of hydrolysis of fluorescein diacetate and the fluorescence quantum yield of fluorescein are dependent on a variety of factors, including the pH and the buffer system, we also explored the effects of varying the pH and the concentration of FDA. While M9 buffer has a pH of approximately 7.0, by adjusting the ratio of phosphate salts, we made variants of M9 buffer with approximately the same total ion concentration, but with pH values ranging from 6.56 to 7.26 in 0.1 pH unit increments. We also examined final FDA concentrations of 1, 2, and 4 ppm. The results of this study are shown in Figure 5.2. Although a high concentration of FDA produced higher recorded fluorescence, there were no real trends observed in the signal-to-noise ratio between variations in either pH or FDA concentration. We
therefore elected to use the standard pH 7.0 M9 buffer, and selected an FDA concentration of 4 ppm, as this level of FDA provided a level of fluorescence high enough for visual monitoring of the 96-well plates.

Lastly, in several initial runs with potential nematicides, we elected to add the FDA solution to the wells at the start of the assay. However, this often led to noisy results, particularly with some compounds that can cause substantial mortality in the nematodes at higher concentrations. We suspect that this excess noise is due to a combination of several factors, including hydrolysis of FDA by esterases present in dying worms and residual esterase activity after cellular death. By waiting four hours to add the FDA solution to the wells, we were able to obtain results that were far more consistent between trials. During the optimization process, we also performed visual checks on the nematodes under a microscope to ensure that the measured fluorescence reflected the observed effects on the nematodes. At very low fluorescence levels, visual observation showed either unhatched eggs or worms that had hatched and quickly died.

Figure 5.1. Plots showing the 24-hour fluorescence of wells containing a nominal number of *C. elegans* eggs. **a.** Fluorescence in arbitrary units, plotted against the number of eggs in each well, with both axes on a log scale. The line is fitted from 50 to 1000 eggs. **b.** A plot of the signal-to-noise ratio, calculated as the ratio of the mean and standard deviation of the fluorescence readings at each egg count. The *x*-axis is square-root transformed. The *x*-values in plot **a** are jittered to improve visibility of clustering.
With our optimized assay in hand, we began screening a variety of compounds found in plant essential oils, as well as some commercially available nematicides for agricultural and medical use. To our surprise, many of the compounds we tested did not show the expected logistic dose-response curve seen in typical mortality studies. Instead, some of these chemicals produced a repeatable hormesis-type effect, wherein the *C. elegans* produced more fluorescein at sublethal concentrations of these compounds that they did at much lower or higher concentrations. Visual observation of these wells showed that many of the nematodes were still alive, but were frequently showing signs of stress, including partial paralysis or atypical movement. To properly fit dose-response curves with a hormetic component, we explored both the Brain-Cousens (BC)\textsuperscript{24} and the Cedargreen-Ritz-Streibig (CRS)\textsuperscript{25} hormesis models. The BC model contains an extra term with a single parameter that allows for hormesis effects, while CRS models contain an extra term containing two parameters that allow for difference sizes and shapes of the hormetic effect in the dose-response curve, and in general, the CRS models provided far better fits.

Figure 5.2. Effects of varying the pH and concentration of FDA on fluorescence (a) and signal-to-noise ratio (b). Each well contained approximately 500 *C. elegans* eggs. Fluorescence values are corrected by subtracting the mean fluorescence of control wells at each pH/FDA combination.
Figure 5.3 shows dose-response curves for eight of the compounds tested. Cinnamaldehyde and geraniol (Figure 5.3a and b) both have dose-response curves close to that of a pure logistic curve, with only minor hormesis effects in the optimal fit.

4-Methoxycinnamaldehyde (Figure 5.3c) exhibits a more significant hormesis effect, wherein sublethal concentrations of this cinnamaldehyde analog increase the level of fluorescence, while cinnamic acid (Figure 5.3d) shows a more substantial hormesis effect. Abamectin, a compound used in both agriculture as a seed treatment, and as an anthelminthic, again shows little hormesis (Figure 5.3e), while both albendazole and nitazoxanide (Figure 5.3f and g) showed substantial hormesis and little-to-no mortality in this assay. Conversely, oxamyl, a carbamate pesticide which has been used as an agricultural nematicide, has a more-traditional dose-response curve, with an EC$_{50}$ around 132 ppm. Because oxamyl has been used a treatment for plant-parasitic nematodes previously, we performed far more replicates with this compound than any other, and were able to test oxamyl in our system at concentrations up to 256 ppm, which was made possible by its high solubility.

By using an optimal CRS or logistic model, as determined by the AIC of the model fit, we obtained the EC$_{50}$ values tabulated in Table 5.1. Of the 19 monoterpenoids and phenylpropanoids screened, only three—cinnamaldehyde, geraniol, and D-limonene—had estimable EC$_{50}$s below 128 ppm. However, ten of these naturally-occurring compounds had a hormetic effect that resulted in fluorescence greater than 10% more than the control. The HC$_{10}$, or the concentration at which this 10% threshold is crossed, is shown in Table 5.2. All six of the cinnamaldehyde analogs tested outperformed cinnamaldehyde for nematicidal activity as determined by EC$_{50}$, with α-bromocinnamaldehyde and 4-methoxycinnamaldehyde as the most effective of these.
Figure 5.3. Dose-response curves for treatments against *C. elegans*. Gray bands represent 95% confidence intervals for the curve. Individual normalized fluorescence values are represented by points, while black line is the best curve fit. The shaded gray area represents the 95% confidence interval of the fit. Treatments are a. cinnamaldehyde, b. geraniol, c. 4-methoxy-cinnamaldehyde, d. cinnamic acid, e. abamectin, f. albendazole, g. nitazoxanide, h. oxamyl.
Table 5.1. EC$_{50}$ values of monoterpenoids and phenylpropanoids, cinnamaldehyde analogs, and agricultural and medical anthelmintic compounds. Listed EC$_{50}$ values were determined using the best dose-response curve fit, which may include hormesis effects, as described in the text. Values in brackets represent the 95% confidence interval.

<table>
<thead>
<tr>
<th>Monoterprenoids and phenylpropanoids</th>
<th>MW (g/mol)</th>
<th>EC$_{50}$ (ppm)</th>
<th>EC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>camphor</td>
<td>152.23</td>
<td>&gt;128</td>
<td>&gt;841</td>
</tr>
<tr>
<td>carvacrol</td>
<td>150.22</td>
<td>&gt;128</td>
<td>&gt;852</td>
</tr>
<tr>
<td>R-carvone</td>
<td>150.22</td>
<td>&gt;128</td>
<td>&gt;852</td>
</tr>
<tr>
<td>cinnamaldehyde</td>
<td>132.16</td>
<td>72.9 [65.6, 81]</td>
<td>551 [496, 613]</td>
</tr>
<tr>
<td>cinnamic acid</td>
<td>148.16</td>
<td>&gt;128</td>
<td>&gt;864</td>
</tr>
<tr>
<td>cinnamyl alcohol</td>
<td>134.17</td>
<td>&gt;128</td>
<td>&gt;954</td>
</tr>
<tr>
<td>citral</td>
<td>154.24</td>
<td>&gt;128</td>
<td>&gt;830</td>
</tr>
<tr>
<td>citronellol</td>
<td>156.27</td>
<td>&gt;128</td>
<td>&gt;819</td>
</tr>
<tr>
<td>eucalyptol</td>
<td>154.25</td>
<td>&gt;128</td>
<td>&gt;830</td>
</tr>
<tr>
<td>eugenol</td>
<td>164.2</td>
<td>&gt;128</td>
<td>&gt;780</td>
</tr>
<tr>
<td>geraniol</td>
<td>154.25</td>
<td>48.5 [41.9, 56.3]</td>
<td>315 [271, 365]</td>
</tr>
<tr>
<td>D-limonene</td>
<td>136.24</td>
<td>96 [61, 151]</td>
<td>704 [448, 1110]</td>
</tr>
<tr>
<td>menthol</td>
<td>156.27</td>
<td>&gt;128</td>
<td>&gt;819</td>
</tr>
<tr>
<td>menthone</td>
<td>154.25</td>
<td>&gt;128</td>
<td>&gt;830</td>
</tr>
<tr>
<td>nerol</td>
<td>154.25</td>
<td>&gt;128</td>
<td>&gt;830</td>
</tr>
<tr>
<td>phenethyl propionate</td>
<td>178.23</td>
<td>&gt;128</td>
<td>&gt;718</td>
</tr>
<tr>
<td>pulegone</td>
<td>152.23</td>
<td>&gt;128</td>
<td>&gt;841</td>
</tr>
<tr>
<td>thymol</td>
<td>150.22</td>
<td>&gt;128</td>
<td>&gt;852</td>
</tr>
<tr>
<td>vanillin</td>
<td>152.15</td>
<td>&gt;128</td>
<td>&gt;841</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cinnamaldehyde Analogs</th>
<th>MW (g/mol)</th>
<th>EC$_{50}$ (ppm)</th>
<th>EC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-(dimethylamino)cinnamaldehyde</td>
<td>175.22</td>
<td>47.4 [41.8, 53.6]</td>
<td>270 [239, 306]</td>
</tr>
<tr>
<td>4-chlorocinnamaldehyde</td>
<td>166.6</td>
<td>43.7 [41.6, 45.8]</td>
<td>262 [250, 275]</td>
</tr>
<tr>
<td>4-fluorocinnamaldehyde</td>
<td>150.15</td>
<td>46.0 [42.8, 49.4]</td>
<td>306 [285, 329]</td>
</tr>
<tr>
<td>4-methoxycinnamaldehyde</td>
<td>162.19</td>
<td>38.9 [36.2, 41.9]</td>
<td>240 [223, 259]</td>
</tr>
<tr>
<td>α-bromocinnamaldehyde</td>
<td>211.06</td>
<td>36.4 [31.9, 41.5]</td>
<td>172 [151, 196]</td>
</tr>
<tr>
<td>methyl styryl ketone</td>
<td>146.19</td>
<td>67.3 [62.0, 73.2]</td>
<td>461 [424, 500]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nematicides and Anthelmintics</th>
<th>MW (g/mol)</th>
<th>EC$_{50}$ (ppm)</th>
<th>EC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>abamectin</td>
<td>873.1</td>
<td>80.6 [67.5, 96.2]</td>
<td>92.3 [77.3, 110]</td>
</tr>
<tr>
<td>carbaryl</td>
<td>201.22</td>
<td>&gt;128</td>
<td>&gt;636</td>
</tr>
<tr>
<td>malathion</td>
<td>330.4</td>
<td>&gt;128</td>
<td>&gt;387</td>
</tr>
<tr>
<td>oxamyl</td>
<td>219.26</td>
<td>132 [128, 136]</td>
<td>603 [586, 621]</td>
</tr>
<tr>
<td>albendazole</td>
<td>265.33</td>
<td>&gt;128</td>
<td>&gt;482</td>
</tr>
<tr>
<td>nitazoxanide</td>
<td>307.28</td>
<td>&gt;128</td>
<td>&gt;417</td>
</tr>
<tr>
<td>pyrantel pamoate</td>
<td>594.68</td>
<td>&gt;128</td>
<td>&gt;215</td>
</tr>
</tbody>
</table>
Amongst the nematicides and anthelmintics, abamectin performed the best, with an EC$_{50}$ of approximately 92.3 μM, the lowest EC$_{50}$ of all compounds screened. Abamectin and related compounds are allosteric modulators of several channels, primarily glutamate-gated chloride channels.\textsuperscript{26-27} The benzimidazole class of anthelmintics, including albendazole, bind to the colchicine-sensitive sites of β-tubulin in the intestinal cells of nematodes, which inhibits microtubule polymerization.\textsuperscript{28} Albendazole (ABZ) has a reported LC$_{50}$ of 0.034 μM seven days after exposure for the common ruminant parasitic nematode \textit{Haemonchus contortus};\textsuperscript{28} and an LC$_{50}$ of 18.4 μM (4.9 ppm) on adult \textit{C. elegans} after seven days of treatment.\textsuperscript{29} In this latter study, an inhibitory effect on egg hatch and larval development was noted at sublethal concentrations. In our assay, ABZ did not result in any measurable EC$_{50}$ after 24 hours. However, the HC$_{10}$ for ABZ was the lowest out of the compounds that were screened at 2.2 μM; additionally, the magnitude of this hormetic effect was much large than for any other compound.

Table 5.2. HC$_{10}$ values for the monoterpenoids, phenylpropanoids, and anthelminthics for which the values exist.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HC$_{10}$ (ppm)</th>
<th>HC$_{10}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>camphor</td>
<td>16.6 [11.6, 23.7]</td>
<td>109 [75.9, 156]</td>
</tr>
<tr>
<td>carvacrol</td>
<td>15.8 [11.6, 21.7]</td>
<td>105 [77, 144]</td>
</tr>
<tr>
<td>R-carvone</td>
<td>16.9 [8.83, 32.4]</td>
<td>113 [58.8, 216]</td>
</tr>
<tr>
<td>cinnamic acid</td>
<td>9.69 [7.19, 13.1]</td>
<td>65.4 [48.6, 88.1]</td>
</tr>
<tr>
<td>cinnamyl alcohol</td>
<td>16.4 [10.4, 25.9]</td>
<td>122 [77.3, 193]</td>
</tr>
<tr>
<td>citral</td>
<td>34.1 [18.9, 61.4]</td>
<td>221 [123, 398]</td>
</tr>
<tr>
<td>menthol</td>
<td>38.7 [7.8, 192]</td>
<td>248 [49.9, 1230]</td>
</tr>
<tr>
<td>menthone</td>
<td>21 [14.5, 30.3]</td>
<td>136 [94, 197]</td>
</tr>
<tr>
<td>nerol</td>
<td>40.7 [21.4, 77.3]</td>
<td>264 [139, 501]</td>
</tr>
<tr>
<td>phenethyl propionate</td>
<td>25.3 [14.7, 43.6]</td>
<td>142 [82.5, 245]</td>
</tr>
<tr>
<td>pulegone</td>
<td>18.5 [10.1, 33.9]</td>
<td>122 [66.4, 223]</td>
</tr>
<tr>
<td>4-methoxycinnamaldehyde</td>
<td>0.967 [0.803, 1.16]</td>
<td>5.96 [4.95, 7.18]</td>
</tr>
<tr>
<td>albendazole</td>
<td>0.586 [0.424, 0.81]</td>
<td>2.21 [1.6, 3.05]</td>
</tr>
<tr>
<td>nitazoxanide</td>
<td>10.8 [7, 16.6]</td>
<td>35 [22.8, 53.9]</td>
</tr>
<tr>
<td>pyrantel pamoate</td>
<td>1.42 [1.09, 1.85]</td>
<td>2.39 [1.83, 3.11]</td>
</tr>
</tbody>
</table>
(see Figure 5.1f), suggesting that although there was not significant *C. elegans* mortality, exposure to ABZ presented a significant stressor to these worms.

Similarly, nitazoxanide (NTZ), which acts through the glutamate-gated chloride channel *avr-14*, was found to have an LC$_{50}$ on the wild-type *C. elegans* N2 strain of approximately 1.7 mM (521 ppm) after 6 days; however, NTZ inhibits growth of larvae to adults with an IC$_{50}$ of 15 μM (4.5 ppm). In our bioassay, we saw little evidence of an estimable EC$_{50}$; however, the calculated HC$_{10}$ was 35 μM, which is on the same order of magnitude as the previously-reported growth inhibitory concentration. Pyrantel (PY) is a selective agonist on nematode nicotinic acetylcholine receptors, and is used as an anthelmintic drug that causes paralysis of parasitic nematodes in the gut, causing the worms to lose their grip and be passed from the digestive tract. PY produced a rather substantial hormetic effect, and the estimated HC$_{10}$ of 2.4 μM was statistically equivalent to ABZ.

Neither carbaryl nor malathion, insecticides from the carbamate and organophosphate classes, respectively, produced any significant hormetic effect, and EC$_{50}$ values could not be estimated for these compounds because of their relatively low toxicity to *C. elegans*. More than half of the monoterpenoids and phenylpropanoids produced a hormetic response at concentrations ranging from approximately 10 to 40 ppm. Although we have not performed any study of the mechanism behind this hormetic effect in our dose-response curve, we suspect that it is the result of an increase in esterase production by the *C. elegans* in response to the presence of a xenobiotic. Soil is often rich in terpenoids and their metabolites, and because *C. elegans* is a soil-dwelling, free-living nematode, it is possible that *C. elegans* has adapted to terpenoids in their environment by increasing the level of metabolic enzymes, including esterases, when these xenobiotics are encountered, resulting in the observed hormesis.
Conclusion

Using fluorescein diacetate as a metabolic indicator, we have developed a low-labor, fluorescence-based assay for the screening of potentially nematicidal compounds against C. elegans. Additionally, this assay frequently allows for the monitoring of nematode stress as detected by an increase in fluorescence above the assay baseline. In screening 19 natural compounds against C. elegans, only cinnamaldehyde, geraniol, and limonene produced EC\textsubscript{50} values below 128 ppm, while more than half of these compounds produced a significant hormetic increase in fluorescence, likely due to metabolic effects. Likewise, the medically significant antinematodal drugs albendazole and pyrantel created a detectable stress response in the worms at concentrations around 2-3 μM after 24 hours. We hope that this assay will prove useful as an easy screening method for other nematicidal screening techniques against C. elegans and other nematodes.

Acknowledgements

This project was by AMVAC Chemical Corporation under Iowa State University grant GR-015523-00001 and by the Iowa Agricultural Experimental Station.

References


**Appendix**

The *drc* package has fully implemented methods for several families of Cedargreen-Ritz-Streibig models. The full model has the formula

\[
\varphi(x|b, c, d, e, f, \alpha) = c + \frac{d - c + f \exp[-x^{-\alpha}]}{1 + \exp[b(\ln(x) - \ln(e))]},
\]

where the two-parameter \( f \exp[-x^{-\alpha}] \) term accounts for a hormesis effect. Specifically, in this family of models, the *drc* package implements five-free-parameter versions of this curve with \( \alpha \) fixed at either 0.25, 0.5, or 1, and four-free-parameter versions of the curve with \( \alpha \) varying as before, and \( c \) also fixed at 0. However, there is not fully-implemented model that contains a freely-varying alpha, either with \( c \) fixed or free.

To allow for model fits with a free parameter \( \alpha \), optimization of the likelihood function

\[
\mathcal{L}(b, c, d, e, f, \alpha|x) = c + \frac{d - c + f \exp[-x^{-\alpha}]}{1 + \exp[b(\ln(x) - \ln(e))]},
\]

would be required.
was performed in R using the `optim` function for nonlinear least squares optimization. The Akaike information criterion (AIC) was determined by calculating 
\[ \text{AIC} = 2k - 2\ln(\hat{L}), \]
where 
\[ k \]
is the number of free parameters in the model, and \( \hat{L} \) is the maximum value of the likelihood function. To calculate EC\(_p\) for a given percentage \( p \), the R function `uniroot` was used to find the zero of the function \( \phi(x) - p \). To calculate the confidence interval of EC\(_p\), the delta method was used to calculate \( \text{Var}(\hat{E}C_p) \), namely, to a first approximation, under the assumption of asymptotic normality,
\[
\text{Var}(\hat{E}C_p) = \nabla \phi(\hat{\theta}, x)^T \text{Var}(\hat{\theta}) \nabla \phi(\hat{\theta}, x),
\]
where \( \theta = (b, c, d, e, f, \alpha)^T \) and \( \text{Var}(\hat{\theta}) = H^{-1}(\hat{\theta}) \) is the inverse of the Hessian of \( \mathcal{L}(\theta, x) \) numerically estimated by `optim`. In the full six-parameter model, we can then determine
\[
d\phi(\hat{\theta}, x) = \nabla \phi(\hat{\theta}, x)^T = \left( \frac{\partial \phi}{\partial b}, \frac{\partial \phi}{\partial c}, \frac{\partial \phi}{\partial d}, \frac{\partial \phi}{\partial e}, \frac{\partial \phi}{\partial f}, \frac{\partial \phi}{\partial \alpha} \right),
\]
where
\[
\frac{\partial \phi(\theta, x)}{\partial b} = \frac{\exp[b(ln(x)+ln(\epsilon))-x^{-\alpha}](ln(x)-ln(\epsilon))((c-d) \exp[x^{-\alpha}-f])}{(x^{b+e})^2},
\]
\[
\frac{\partial \phi(\theta, x)}{\partial c} = \frac{x^b}{x^{b+e}+\epsilon^b},
\]
\[
\frac{\partial \phi(\theta, x)}{\partial d} = \frac{x^{b+e}}{\epsilon^b},
\]
\[
\frac{\partial \phi(\theta, x)}{\partial e} = \frac{-e^b \cdot (c-d) \exp[x^{-\alpha}-f] \cdot x^b \exp[-x^{-\alpha}]}{(x^{b+e})^2},
\]
\[
\frac{\partial \phi(\theta, x)}{\partial f} = \frac{e^b \exp[-x^{-\alpha}]}{x^{b+e}+\epsilon^b},
\]
\[
\frac{\partial \phi(\theta, x)}{\partial \alpha} = \frac{f \cdot e^b \cdot x^{-\alpha} \exp[-x^{-\alpha}] \ln(x)}{x^{b+e}+\epsilon^b}.
\]
From this, we can then fit four-, five-, or six-parameter CRS models with free \( \alpha \). In the five-parameter model, \( c \) is fixed at 0, while in the four-parameter model, \( d \) is also fixed at 1, in both cases with appropriate changes to the gradient for curve optimization.

Once \( \text{Var}(\hat{E}C_p) \) has been determined, the confidence interval for an effective concentration can be calculated by determining the EC\(_p\), converting to the log-scale and using the Student’s \( t \)-distribution, followed by transforming back from the log scale.
Despite the further implementation of the six-parameter CRS (CRS6) model, no set of data obtained in this experiment yielded a better model fit with the full CRS6 model compared to the four- and five-parameter models using the Akaike information criterion (AIC) as a means of model selection.
CHAPTER 6. ANALOGS OF CINNAMALDEHYDE AS NEMATICIDES: STYRYL KETONES, STYRYL SULFIDES, B-NITROSTYRENES, AND AZOLES

James S. Klimavicz,1,2* Jefferson O. Barizon,3 Claire M. Pouliot,1
Gregory L. Tylka,3 and Joel R. Coats1

1Department of Entomology, Iowa State University, Ames, IA, 50011
2Department of Chemistry, Iowa State University, Ames, IA, 50011
3Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to the Journal of Agricultural and Food Chemistry.

Abstract

Background

By damaging and destroying agricultural crops, plant-parasitic nematodes present a substantial global economic burden; as an example, soybean cyst nematode (*Heterodera glycines, SCN*) is of considerable concern in soybean-producing areas as it can weaken plants and severely reduce crop yields. While agricultural practices can reduce soil nematode loads, chemical nematicides are also important for managing some of these nematode pests. However, current chemical treatments for nematodes is limited—methyl bromide, a soil fumigant that was once commonly used for nematodes and plant-pathogenic bacteria and fungi, is no longer approved for most agricultural uses, and other nematicides suffer for lower efficacy, higher toxicity, or higher cost. Given the need for new chemical nematicides, we turned to cinnamaldehyde, a natural component of cinnamon extract that has previously been shown to

* JSK designed, synthesized, purified, and characterized all compounds for testing and wrote the manuscript, JOB performed the SCN bioassay testing, CMP performed the *C. elegans* bioassay tests, GLT assisted with bioassay design and implementation, and JRC helped formulate the project and revised the manuscript.
have nematicidal properties, to lead a biorational approach to new compounds that may have improved efficacy against nematodes.

Results

Sixty-four compounds, including cinnamaldehyde and six derivatives thereof, six β-nitrostyrenes, 32 styryl ketones, and 12 azole-containing cinnamaldehyde analogs were screened against SCN eggs at 100 ppm in a 15-day assay to determine the ability of these compounds to inhibit egg hatch; 44 of these compounds were also screened in a fluorescence-based *C. elegans* egg hatch assay. Cinnamaldehyde and nitrostyrene compounds were generally very active against SCN and *C. elegans*, as were most of the styryl ketones, with some styryl ketones inhibiting more than 80% of egg hatch compared to the solvent control. Several azoles were significant SCN hatch inhibitors, and two of these azoles, an isothiazole and a pyrazole, caused physiological responses in *C. elegans* at sub-μM concentrations.

Conclusion

The biorational approach to developing cinnamaldehyde analogs successfully led to many modulators of SCN egg hatch, both amongst the styryl ketones and azoles. Correlation between the SCN hatch modulation data and the dose-response data obtained from *C. elegans* was poor; however, *C. elegans* was useful as a binary predictor in determining which compounds would likely modulate SCN hatch rates.

**Keywords**: nematicides, soybean cyst nematode, *Heterodera glycines*, cinnamaldehyde, *C. elegans*, biorational

Introduction

Plant-parasitic nematodes are responsible for substantial reduction of the annual yields in many crops.\(^1\)\(^2\) The soybean cyst nematode (SCN, *Heterodera glycines*), for example, is a destructive nematode introduced into the southeast United States from Asia, and first detected in
the 1950s; SCN has since become widespread in soybean-producing areas in the eastern US.\(^3\)
SCN can cause crop losses well in excess of 30%, and over the five year period of 2010 – 2014, an estimated 617 million bushels of soybeans, worth approximately $7.5 billion, was lost to SCN damage.\(^4\) Other plant-parasitic nematodes, including the root-knot (*Meloidogyne* spp.), root lesion (*Pratylenchus* spp.), and foliar (*Aphelenchoides* spp.) nematodes, are of significant agricultural concern to a wide variety of crops;\(^2\) however, commodity crops have historically not been treated for plant-parasitic nematode diseases, as treatments are frequently cost-prohibitive compared to the market value of these crops.

Many of the chemical treatments that have been used as nematicides are soil sterilants, including alkylating agents and chloropicrin. In particular, methyl bromide enjoyed significant popularity as a nematicide and soil sterilant before its use in most applications was prohibited due to its status as a potent ozone-depleting chemical.\(^5\) Although chloropicrin and other fumigants, including 1,3-dichloropropene, chloropicrin, and dazomet (which produces methyl isothiocyanate on decomposition in soil) are still permitted for agricultural use, the compounds are less effective and more expensive than methyl bromide.\(^6\) Other pesticides registered for agricultural use against nematodes include aldicarb, oxamyl, and abamectin, but off-target toxicity and cost remain important considerations—the choice of chemical treatment for plant-parasitic nematodes is highly dependent on the value of the crop. The low price of soybeans on the commodity market has limited most SCN management to non-chemical integrated pest management methods, including crop rotation and the use of SCN-resistant strains of soybeans. However, SCN eggs can survive in the soil for years, and weeds can serve as hosts for SCN, complicating crop-rotation efforts, and some populations of SCN are developing a tolerance for the resistant soybean varieties.\(^7\-\(^9\)
The ongoing economic burden of plant-parasitic nematodes, continues to drive the search for new technologies to control these pests. Plant essential oils and many of their components have been screened as potential nematicides.\textsuperscript{10-11} Cinnamaldehyde, a biocidal compound found in high concentration in the essential oils derived from the bark of \textit{Cinnamomum spp.}, is a readily-available compound with well-established activity against various fungi,\textsuperscript{12-13} bacteria,\textsuperscript{14-15} insects,\textsuperscript{16} and plant-parasitic nematodes.\textsuperscript{17-18} Given the efficacy of cinnamaldehyde as a nematicide, we therefore chose to develop several series of analogs and related compounds to optimize the nematicidal activity.

Because of the widespread economic concern of SCN, this nematode was chosen for screening. For much of their lives, \textit{M. glycines} nematodes are protected from most pesticides as some of the SCN eggs in the soil are encapsulated in durable cysts, and recently-hatched J2 larvae are only exposed for a short period of time as they rapidly seek the roots of an acceptable host plant.\textsuperscript{19} To determine the effects of exposure to these compounds at a vulnerable stage in the SCN life cycle, we elected to use an egg-hatch inhibition assay on eggs not protected by cysts. As a point of comparison between SCN and other nematodes, we also determined the toxicity of these cinnamaldehyde analogs on \textit{Caenorhabditis elegans}, a common model organism, using a previously-described fluorescence-based screening assay (see Chapter 5).

\textbf{Materials and Methods}

\textbf{General information}

All solvents were purchased from Fisher Scientific and used as received. Cinnamaldehyde and β-nitrostyrene analogs and methyl styryl ketone (3a) were purchased from either Sigma-Aldrich or TCI and were used as received. All other reagents were purchased from TCI, Alfa Aesar, Oakwood Chemicals, Chem-Impex, or Maybridge and were used as received. Reaction products were visualized via TLC under UV light or by staining with KMnO$_4$ or
cerium(IV) ammonium molybdenate stain. NMR spectra were obtained using Bruker MR 400 MHz, Avance NEO 400 MHz, or Avance III 600 MHz spectrometers in the Iowa State University Chemical Instrumentation Facility. Chemical shifts are reported in ppm relative to the residual deuterated solvent peak (CDCl$_3$: 7.26 ppm for $^1$H and 77.16 ppm for $^{13}$C; DMSO-$d_6$: 2.50 for $^1$H and 39.52 ppm for $^{13}$C). HRMS analysis was performed using positive ion mode electrospray ionization (ESI+) on a quadrupole time-of-flight mass spectrometer (Agilent QTOF 6540). The constant infusion of calibrant (masses: 121.0508 and 922.0098) allowed for accurate mass measurement. Melting points are uncorrected and were obtained on Stuart SMP30 melting point apparatus using a temperature ramp rate of 5 ºC min$^{-1}$.

**Analog synthesis**

**General synthesis of styryl ketones (GS1):** An aryl carboxaldehyde (5 mmol) and a methyl ketone (7.5 mmol) were dissolved in ethanol (25 mL), and 1 M sodium hydroxide (10 mL) was added in one portion. The reaction was stirred for 18 hours at 22 ºC, and then water (50 mL) was added. If a product precipitated, the mixture was cooled to 0 ºC, and the crude material was collected by filtration, washing with water, and the crude product was recrystallized from hot 95% ethanol. If the product did not precipitate out as a solid, the mixture was extracted twice with ethyl acetate (20 mL each), and the combined organic layers were washed with water (20 mL), and then brine (25 mL), before drying the organic layer over anhydrous sodium sulfate. The ethyl acetate was removed under vacuum, and the crude product was either recrystallized or purified by column chromatography on silica. Any deviations from this procedure are noted.

**4-Methoxystyryl methyl ketone (3b)** Synthesized according to GS1 from 4-anisaldehyde and acetone. Purified by column chromatography (90:10 to 75:25 hexane:ethyl acetate). Light yellow solid. (2.42 g, 69%). mp 69 – 70 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.53 – 7.46 (m, 2H), 7.46 (d, $J$ = 16.2 Hz, 1H), 6.96 – 6.83 (m, 2H), 6.60 (d, $J$ = 16.2 Hz, 1H), 3.84 (s,
116

$^1$H NMR (101 MHz, CDCl$_3$) δ 198.5, 161.7, 143.4, 130.1, 127.2, 125.1, 114.6, 55.5, 27.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calced for C$_{11}$H$_{13}$O$_2^+$ 177.0910; found: 177.0910.

4-Methoxystyryl isopropyl ketone (3c) Synthesized according to GS1 from 4-chlorobenzaldehyde and methyl isopropyl ketone on a 50 mmol scale. Purified by column chromatography (20:80 ethyl acetate:hexane). Pale yellow liquid (6.21 g, 61%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.56 (d, $J$ = 16.0 Hz, 1H), 7.52 – 7.46 (m, 2H), 6.92 – 6.86 (m, 2H), 6.69 (d, $J$ = 16.0 Hz, 1H), 3.82 (s, 3H), 2.91 (hept, $J$ = 6.9 Hz, 1H), 1.16 (d, $J$ = 6.9 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 203.9, 161.5, 142.2, 130.0, 127.4, 122.3, 114.4, 55.5, 55.4, 39.2, 18.7. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{17}$O$_2^+$ 205.1223; found: 205.1223.

4-Chlorostyryl t-butyl ketone (3d) Synthesized according to GS1 from 4-chlorobenzaldehyde and methyl t-butyl ketone on a 20 mmol scale. White acicular crystals (1.71 g, 51%). mp 84 – 85 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.56 (d, $J$ = 15.6 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.39 – 7.31 (m, 2H), 7.09 (d, $J$ = 15.6 Hz, 1H), 1.22 (s, 9H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 204.1, 141.6, 136.1, 133.6, 129.6, 129.2, 121.3, 43.4, 26.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{16}$ClO$^+$ 223.0884; found: 220.0883.

4-Bromostyryl t-butyl ketone (3e) Synthesized according to GS1 from 4-bromobenzaldehyde and methyl t-butyl ketone on a 20 mmol scale. White acicular crystals (2.86 g, 71%). mp 96 – 97 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60 (d, $J$ = 15.6 Hz, 1H), 7.54 – 7.47 (m, 2H), 7.47 – 7.39 (m, 2H), 7.10 (d, $J$ = 15.6 Hz, 1H), 1.22 (s, 9H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 204.1, 141.7, 134.0, 132.2, 129.8, 124.5, 121.4, 43.4, 26.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{16}$BrO$^+$ 267.0379; found: 267.0376.
Styryl cyclopropyl ketone (3f) Synthesized according to GS1 from benzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Iridescent white plates (2.16 g, 63%). mp 54 – 55 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.61 (d, $J = 16.1$ Hz, 1H), 7.58 – 7.53 (m, 2H), 7.42 – 7.36 (m, 3H), 6.87 (d, $J = 16.1$ Hz, 1H), 2.25 (tt, $J = 7.8$, 4.5 Hz, 1H), 1.20 – 1.12 (m, 2H), 1.03 – 0.91 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.1, 142.1, 134.7, 130.4, 129.0, 128.3, 126.5, 19.7, 11.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{13}$O$^+$ 173.0961; found: 173.0961.

3-Fluorostyryl cyclopropyl ketone (3g) Synthesized according to GS1 from 3-fluorobenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Pale yellow oil (2.13 g, 56%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.55 (d, $J = 16.1$ Hz, 1H), 7.40 – 7.30 (m, 2H), 7.29 – 7.22 (m, 1H), 7.12 – 7.03 (m, 2H), 6.85 (d, $J = 16.1$ Hz, 1H), 2.23 (tt, $J = 7.8$, 4.5 Hz, 1H), 1.22 – 1.12 (m, 2H), 1.00 (dt, $J = 8.1$, 3.5 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 199.8, 163.0 (d, $J = 246.8$ Hz), 140.5 (d, $J = 2.8$ Hz), 137.0 (d, $J = 7.6$ Hz), 130.4 (d, $J = 8.3$ Hz), 127.4, 124.3 (d, $J = 3.0$ Hz), 117.1 (d, $J = 21.5$ Hz), 114.4 (d, $J = 21.9$ Hz), 19.9, 11.5. $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -112.54 (td, $J = 9.1$, 5.4 Hz). HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{12}$FO$^+$ 191.0867; found: 191.0867.

4-Fluorostyryl cyclopropyl ketone (3h) Synthesized according to GS1 from 4-fluorobenzaldehyde and methyl cyclopropyl ketone on a 10 mmol scale. Pale yellow oil (1.01 g, 53%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.57 (d, $J = 16.1$ Hz, 1H), 7.58 – 7.52 (m, 2H), 7.13 – 7.03 (m, 2H), 6.80 (d, $J = 16.1$ Hz, 1H), 2.23 (tt, $J = 7.8$, 4.5 Hz, 1H), 1.15 (dt, $J = 4.6$, 3.4 Hz, 2H), 0.98 (dt, $J = 8.1$, 3.5 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.0, 164.0 (d, $J = 251.5$ Hz), 140.8, 131.1 (d, $J = 3.3$ Hz), 130.3 (d, $J = 8.6$ Hz), 126.2 (d, $J = 2.3$ Hz), 116.2 (d, $J = 22.0$ Hz), 19.9, 11.5. $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -109.5 (tt, $J = 8.7$, 5.4 Hz). HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{12}$FO$^+$ 191.0867; found: 191.0867.
4-Chlorostyryl cyclopropyl ketone (3i) Synthesized according to GS1 from 4-
chlorobenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Purified by
recrystallization. White crystals (3.72 g, 90%). mp 65 – 66 ºC. ¹H NMR (400 MHz, CDCl₃) δ
7.54 (d, J = 16.1 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.39 – 7.31 (m, 2H), 6.83 (d, J = 16.1 Hz, 1H),
2.22 (tt, J = 7.8, 4.5 Hz, 1H), 1.18 – 1.12 (m, 2H), 1.01 – 0.95 (m, 2H). ¹³C NMR (101 MHz,
CDCl₃) δ 199.8, 140.4, 136.2, 133.2, 129.4, 129.2, 126.8, 19.8, 11.5. HRMS (+ESI-QTOF) m/z:
[M+H]+ calcd for C₁₂H₁₂ClO+ 207.0571; found: 207.0573.

4-Bromostyryl cyclopropyl ketone (3j) Synthesized according to GS1 from 4-
bromobenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. White crystals (4.62 g,
92%). mp 78 – 80 ºC. ¹H NMR (400 MHz, CDCl₃) δ 7.52 (d, J = 16.1 Hz, 2H), 7.52 – 7.47 (m,
2H), 7.45 – 7.37 (m, 2H), 6.85 (d, J = 16.1 Hz, 1H), 2.22 (tt, J = 7.8, 4.5 Hz, 1H), 1.15 (m, 2H),
0.98 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 199.9, 140.6, 133.7, 132.2, 129.7, 126.9, 124.6,
19.9, 11.6. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C₁₂H₁₁BrO+ 251.0066; found: 251.0066.

2,4-Dichlorostyryl cyclopropyl ketone (3k) Synthesized according to GS1 from 2,4-
dichlorobenzaldehyde and methyl cyclopropyl ketone on a 10 mmol scale. White crystals (2.07
g, 86%). mp 83 – 85 ºC. ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, J = 16.2 Hz, 1H), 7.58 (d, J =
8.5 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.26 (dd, J = 8.5, 2.1 Hz, 1H), 6.79 (d, J = 16.1 Hz, 1H),
2.28 (tt, J = 7.8, 4.5 Hz, 1H), 1.21 – 1.13 (m, 2H), 1.06 – 0.94 (m, 2H). ¹³C NMR (101 MHz,
CDCl₃) δ 199.8, 136.6, 136.4, 135.9, 131.7, 130.1, 129.3, 128.4, 127.7, 19.6, 11.8. HRMS
(+ESI-QTOF) m/z: [M+H]+ calcd for C₁₂H₁₁Cl₂O+ 241.0181; found: 241.0182.

3,4-Dichlorostyryl cyclopropyl ketone (3l) Synthesized according to GS1 from 3,4-
dichlorobenzaldehyde and methyl cyclopropyl ketone. White crystals (.939 g, 78%). mp 79 – 81
ºC. ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, J = 2.0 Hz, 1H), 7.47 (d, J = 16.0 Hz, 1H), 7.45 (d, J
\[ \delta = 8.3 \text{ Hz, 1H}, 7.37 (\text{dd, } J = 8.4, 2.0 \text{ Hz, 1H}), 6.84 (\text{d, } J = 16.1 \text{ Hz, 1H}), 2.21 (\text{tt, } J = 7.8, 4.5 \text{ Hz, 1H}), 1.21 - 1.12 (\text{m, 2H}), 1.06 - 0.93 (\text{m, 2H}). \]

\[ ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta 199.7, 139.2, 134.9, 134.3, 133.3, 131.0, 129.8, 127.8, 127.4, 20.2, 11.8. \]

HRMS (+ESI-QTOF) \( m/z: [\text{M+H}]^+ \text{ calcd for C}_{12}\text{H}_{11}\text{Cl}_2\text{O}^+ 241.0181; \text{ found: 241.0181}. \]

**4-Bromo-2-fluorostyryl cyclopropyl ketone (3m)** Synthesized according to GS1 from 4-bromo-2-fluorobenzaldehyde and methyl cyclopropyl ketone. Recrystallized from hexane at -20 °C. Light tan crystalline solid (2.37 g, 88%). mp 72 – 73 °C. \( ^{1}H \text{ NMR (400 MHz, CDCl}_3) \delta 7.60 (\text{d, } J = 16.3 \text{ Hz, 1H}), 7.39 (\text{t, } J = 8.2 \text{ Hz, 1H}), 7.30 - 7.21 (\text{m, 2H}), 6.88 (\text{d, } J = 16.3 \text{ Hz, 1H}), 2.20 (\text{tt, } J = 7.9, 4.5 \text{ Hz, 1H}), 1.16 - 1.08 (\text{m, 2H}), 1.00 - 0.89 (\text{m, } J = 8.2, 3.5 \text{ Hz, 2H}). \]

\[ ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta 199.9, 161.1 (\text{d, } J = 258.3 \text{ Hz}), 133.3 (\text{d, } J = 2.6 \text{ Hz}), 129.9 (\text{d, } J = 3.7 \text{ Hz}), 129.0 (\text{d, } J = 5.9 \text{ Hz}), 128.1 (\text{d, } J = 3.6 \text{ Hz}), 124.5 (\text{d, } J = 9.9 \text{ Hz}), 122.0 (\text{d, } J = 11.9 \text{ Hz}), 120.0 (\text{d, } J = 25.2 \text{ Hz}), 19.9, 11.8. \]

\[ ^{19}\text{F NMR (376 MHz, CDCl}_3) \delta -111.9 (\text{dd, } J = 10.1, 7.6 \text{ Hz}). \]

HRMS (+ESI-QTOF) \( m/z: [\text{M+H}]^+ \text{ calcd for C}_{11}\text{H}_{11}\text{BrFO}^+ 268.9972; \text{ found: 268.9971}. \]

**4-Chloro-2-fluorostyryl cyclopropyl ketone (3n)** Synthesized according to GS1 from 4-chloro-2-fluorobenzaldehyde benzaldehyde and methyl cyclopropyl ketone. Recrystallized from hexane at -20 °C; recovery is poor due to high solubility of product in most organic solvents. White crystalline solid (0.172 g, 22%). mp 50 – 51 °C. \( ^{1}H \text{ NMR (400 MHz, CDCl}_3) \delta 7.94 (\text{d, } J = 16.2 \text{ Hz, 1H}), 7.65 (\text{dd, } J = 8.8, 6.0 \text{ Hz, 1H}), 7.17 (\text{dd, } J = 8.4, 2.6 \text{ Hz, 1H}), 7.02 (\text{dddd, } J = 8.6, 7.8, 2.6, 0.6 \text{ Hz, 1H}), 6.76 (\text{dd, } J = 16.1, 0.6 \text{ Hz, 1H}), 2.28 (\text{tt, } J = 7.8, 4.5 \text{ Hz, 1H}), 1.21 - 1.09 (\text{m, 2H}), 1.09 - 0.93 (\text{m, 2H}). \]

\[ ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta 199.9, 163.3 (\text{d, } J = 254.4 \text{ Hz}), 136.7 (\text{d, } J = 1.5 \text{ Hz}), 136.3 (\text{d, } J = 10.5 \text{ Hz}), 129.5 (\text{d, } J = 3.8 \text{ Hz}), 129.0 (\text{d, } J = 9.0 \text{ Hz}), 128.9 (\text{d, } J = 2.1 \text{ Hz}), 117.6 (\text{d, } J = 24.8 \text{ Hz}), 114.9 (\text{d, } J = 21.7 \text{ Hz}), 19.5, 11.7. \]

\[ ^{19}\text{F NMR (376 MHz,} \]
CDCl$_3$ $\delta$ -108.11 (td, $J = 8.1, 6.0$ Hz). HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{11}$ClF$^+$ 225.0477; found: 225.0475.

4-(Trifluoromethyl)styryl cyclopropyl ketone (3o) Synthesized according to GS1 from 4-(trifluoromethyl)benzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. White acicular crystals (2.53 g, 53%). mp 54 – 55 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.68 – 7.62 (m, 4H), 7.59 (d, $J = 16.1$ Hz, 1H), 6.92 (d, $J = 16.1$ Hz, 1H), 2.25 (tt, $J = 7.8, 4.5$ Hz, 1H), 1.20 – 1.14 (m, 2H), 1.04 – 0.98 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 199.74, 139.91, 138.14 (q, $J = 1.3$ Hz), 131.68 (q, $J = 32.8$ Hz), 128.41, 128.34, 125.84 (q, $J = 3.8$ Hz), 123.82 (q, $J = 272.1$ Hz), 19.98, 11.71. $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.9. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{12}$F$_3$O$^+$ 241.0835; found: 241.0835.

4-Nitrostyryl cyclopropyl ketone (3p) Synthesized according to GS1 from 4-nitrobenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Pale yellow crystals (3.18 g, 73%). mp 120 – 121 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.28 – 8.20 (m, 2H), 7.74 – 7.66 (m, 2H), 7.60 (d, $J = 16.1$ Hz, 1H), 6.97 (d, $J = 16.1$ Hz, 1H), 2.25 (tt, $J = 7.8, 4.6$ Hz, 1H), 1.23 – 1.14 (m, 2H), 1.09 – 0.97 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 199.6, 148.5, 141.1, 138.9, 129.9, 128.9, 124.3, 20.4, 12.1. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{12}$NO$_3$ $^+$ 218.0812; found: 218.0811.

4-Cyanostyryl cyclopropyl ketone (3q) Pale yellow crystals. Synthesized according to GS1 from 4-cyanobenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Beige solid forms upon standing; collected solid was recrystallized from ethanol to yield light tan crystals (1.63, 42%). mp 92 – 94 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.72 – 7.60 (m, 4H), 7.56 (d, $J = 16.1$ Hz, 1H), 6.93 (d, $J = 16.1$ Hz, 1H), 2.24 (tt, $J = 7.8, 4.5$ Hz, 1H), 1.21 – 1.15 (m, 2H), 1.06 – 0.97 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 199.7, 139.4, 139.2, 132.8, 129.3, 128.7, 118.5,
113.4, 20.3, 12.0. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C_{13}H_{12}NO + 198.0913; found: 198.0914.

**4-Methylstyril cyclopropyl ketone (3r)** Synthesized according to GS1 from 4-tolualdehyde and methyl cyclopropyl ketone on a 20 mmol scale. White crystals (2.97 g, 80%). mp 78 – 80 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.60 (d, $J$ = 16.1 Hz, 1H), 7.50 – 7.43 (m, 2H), 7.20 (d, $J$ = 7.9 Hz, 2H), 6.84 (d, $J$ = 16.1 Hz, 1H), 2.38 (s, 3H), 2.24 (tt, $J$ = 7.8, 4.5 Hz, 1H), 1.20 – 1.11 (m, 2H), 1.03 – 0.92 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.2, 142.2, 140.9, 132.0, 129.8, 128.4, 125.6, 21.6, 19.7, 11.4. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C$_{13}$H$_{15}$O + 187.1117; found: 187.1116/

**4-Methoxystyril cyclopropyl ketone (3s)** Synthesized according to GS1 from 4-anisaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Purified by recrystallization. Light yellow acicular crystals. 3.64 g, 90% yield. mp 71 – 72 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.58 (d, $J$ = 16.1 Hz, 1H), 7.55 – 7.47 (m, 2H), 6.95 – 6.87 (m, 2H), 6.76 (d, $J$ = 16.0 Hz, 1H), 3.84 (s, 3H), 2.22 (tt, $J$ = 7.8, 4.6 Hz, 1H), 1.17 – 1.11 (m, 2H), 0.98 – 0.92 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.1, 161.6, 141.9, 130.1, 127.5, 124.4, 114.5, 55.5, 19.7, 11.3. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C$_{13}$H$_{15}$O$_2$ + 203.1067; found: 203.1067.

**4-Ethoxystyril cyclopropyl ketone (3t)** Synthesized according to GS1 from 4-ethoxybenzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale, with the reaction temperature held at 50 °C. White flakes (3.78 g, 87%). mp 82 – 84 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.58 (d, $J$ = 16.0 Hz, 1H), 7.54 – 7.47 (m, 2H), 6.94 – 6.85 (m, 2H), 6.76 (d, $J$ = 16.0 Hz, 1H), 4.06 (q, $J$ = 7.0 Hz, 2H), 2.22 (tt, $J$ = 7.8, 4.6 Hz, 1H), 1.43 (t, $J$ = 7.0 Hz, 3H), 1.18 – 1.10 (m, 2H), 1.01 – 0.90 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.1, 161.0, 142.0, 130.1,
127.3, 124.3, 115.0, 63.7, 19.6, 14.9, 11.2. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{14}H_{17}O_{2}^+ 217.1223; found: 217.1223.

3,4-Dimethoxystyril cyclopropyl ketone (3u) Synthesized according to GS1 from 3,4-dimethoxybenzaldehyde and methyl cyclopropyl ketone on a 10 mmol scale. Light yellow microcrystals after recrystallization from toluene/heptane (1.64 g, 71%). mp 92 – 94 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.56 (d, \(J = 16.0\) Hz, 1H), 7.14 (dd, \(J = 8.3, 2.0\) Hz, 1H), 7.09 (d, \(J = 2.0\) Hz, 1H), 6.87 (d, \(J = 8.3\) Hz, 1H), 6.76 (d, \(J = 16.0\) Hz, 1H), 3.91 (s, 3H), 3.91 (s, 3H), 2.24 (tt, \(J = 7.8, 4.5\) Hz, 1H), 1.18 – 1.10 (m, 2H), 1.01 – 0.90 (m, 2H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 200.1, 151.3, 149.3, 142.2, 127.7, 124.7, 123.1, 111.1, 109.7, 56.1, 56.0, 19.5, 11.3. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{14}H_{17}O_{3}^+ 233.1172; found: 233.1175.

3-Ethoxy-4-methoxystyril cyclopropyl ketone (3v) Synthesized according to GS1 from 3-ethoxy-4-methoxybenzaldehyde and methyl cyclopropyl ketone on an 8 mmol scale. Light yellow prisms after recrystallization from toluene/heptane (1.31 g, 81%). mp 121 – 122 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.56 (d, \(J = 16.0\) Hz, 1H), 7.14 (dd, \(J = 8.3, 2.1\) Hz, 1H), 7.10 (d, \(J = 2.0\) Hz, 1H), 6.88 (d, \(J = 8.3\) Hz, 1H), 6.74 (d, \(J = 16.0\) Hz, 1H), 4.14 (q, \(J = 7.0\) Hz, 2H), 3.91 (s, 3H), 2.24 (tt, \(J = 7.8, 4.6\) Hz, 1H), 1.49 (t, \(J = 7.0\) Hz, 3H), 1.19 – 1.11 (m, 2H), 1.00 – 0.92 (m, 2H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 200.1, 151.6, 148.7, 142.3, 127.7, 124.6, 123.0, 111.4, 111.2, 64.5, 56.1, 19.5, 14.9, 11.3. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{15}H_{19}O_{3}^+ 247.1329; found: 247.1327.

3,4-Methylenedioxyxystyril cyclopropyl ketone (3w) Synthesized according to GS1 from piperonal and methyl cyclopropyl ketone on a 10 mmol scale. Pale yellow solid (1.43 g, 66%). mp 87 – 88 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.53 (d, \(J = 15.9\) Hz, 1H), 7.08 (d, \(J = 1.7\) Hz, 1H), 7.05 (dd, \(J = 8.0, 1.7\) Hz, 1H), 6.82 (d, \(J = 8.0\) Hz, 1H), 6.72 (d, \(J = 15.9\) Hz, 1H), 6.01 (s,
1-(2,3-Dihydrobenzofuran-5-yl)vin2-yl cyclopropyl ketone (3x) Synthesized according to GS1 from 2,3-dihydrobenzofuran5-carboxaldehyde and methyl cyclopropyl ketone.
Recrystallized at -20 ºC from hexane/diethyl ether to yield light tan prisms (0.671 g, 63%). mp 96 – 98 ºC. 

1H NMR (400 MHz, CDCl3) δ 7.57 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 1.7 Hz, 1H), 7.34 (dd, J = 8.3, 1.9 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 16.0 Hz, 1H), 4.63 (t, J = 8.7 Hz, 2H), 3.24 (t, J = 8.7 Hz, 2H), 2.21 (tt, J = 7.9, 4.5 Hz, 1H), 1.18 – 1.10 (m, 2H), 1.01 – 0.90 (m, 2H). 

13C NMR (101 MHz, CDCl3) δ 200.1, 162.5, 142.4, 130.0, 128.3, 127.6, 124.8, 123.8, 109.9, 72.0, 29.4, 19.7, 11.2. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C14H14O2 215.1067; found: 215.1066.

4-(Methylthio)styryl cyclopropyl ketone (3y) Synthesized according to GS1 from 4-(methylthio)benzaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Bright yellow crystals (1.89 g, 43%). mp 67 – 68 ºC. 

1H NMR (400 MHz, CDCl3) δ 7.56 (d, J = 16.1 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.25 – 7.18 (m, 2H), 6.83 (d, J = 16.1 Hz, 1H), 2.50 (s, 3H), 2.23 (tt, J = 7.8, 4.6 Hz, 1H), 1.21 – 1.11 (m, 2H), 1.03 – 0.92 (m, 2H). 

13C NMR (101 MHz, CDCl3) δ 200.1, 142.1, 141.6, 131.3, 128.8, 126.1, 125.5, 19.8, 15.3, 11.5. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C13H13O2 219.0838; found: 219.0836.

4-(Methylsulfonyl)styryl cyclopropyl ketone (3z) Synthesized according to GS1 from 4-(methylsulfonyl)benzaldehyde and methyl cyclopropyl ketone on a 10 mmol scale. Pale yellow crystals (1.51 g, 60%). mp 121 – 123 ºC. 

1H NMR (400 MHz, CDCl3) δ 7.99 – 7.91 (m, 2H), 7.76 – 7.69 (m, 2H), 7.59 (d, J = 16.1 Hz, 1H), 6.96 (d, J = 16.1 Hz, 1H), 3.06 (s, 3H), 2.24 (tt, J = 7.8, 4.6 Hz, 1H). 

13C NMR (101 MHz, CDCl3) δ 200.0, 149.8, 148.5, 141.9, 129.3, 125.0, 124.7, 108.8, 106.7, 101.7, 19.8, 11.3. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C13H13O3 217.0859; found: 217.0860.
4-(Dimethylamino)styryl cyclopropyl ketone (3aa) Synthesized according to GS1 from 4-(dimethylamino)benzaldehyde and methyl cyclopropyl ketone, modified such that isopropanol was used instead of ethanol, and the reaction was heated to 70 °C for 18 hours. Bright yellow plates (1.28 g, 60%). mp 138 – 140 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.58 (d, $J = 15.9$ Hz, 1H), 7.50 – 7.42 (m, 2H), 6.70 (d, $J = 15.9$ Hz, 1H), 6.69 – 6.64 (m, $J = 1.8$ Hz, 2H), 3.02 (s, 5H), 2.22 (tt, $J = 7.8$, 4.6 Hz, 1H), 1.17 – 1.08 (m, 2H), 0.98 – 0.87 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.0, 151.9, 142.9, 130.2, 122.4, 121.8, 111.9, 40.2, 19.4, 10.9. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{14}$H$_{18}$NO $^+$ 216.1383; found: 216.1381.

4-Morpholinostyryl cyclopropyl ketone (3bb) Synthesized according to GS1 from 4-morpholinobenzaldehyde and methyl cyclopropyl ketone on a 10 mmol scale, modified such that isopropanol was used instead of ethanol, and the reaction was heated to 50 °C for 18 hours. Bright yellow platelets after recrystallization from heptane (1.64 g, 64%). mp 111 – 113 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.56 (d, $J = 16.0$ Hz, 1H), 7.52 – 7.43 (m, 2H), 6.92 – 6.83 (m, 2H), 6.74 (d, $J = 16.0$ Hz, 1H), 3.88 – 3.81 (m, 4H), 3.28 – 3.21 (m, 4H), 2.22 (tt, $J = 7.8$, 4.6 Hz, 1H), 1.17 – 1.09 (m, 2H), 0.99 – 0.88 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 200.1, 152.7, 142.1, 129.9, 125.7, 123.4, 114.8, 66.8, 48.2, 19.5, 11.1. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{16}$H$_{20}$NO$_2$ $^+$ 258.1489; found: 258.1488.

1-(Pyridin-4-yl)vin-2-yl cyclopropyl ketone (3cc) Synthesized according to GS1 from 4-pyridinecarboxaldehyde and methyl cyclopropyl ketone on a 20 mmol scale. Light pink, fibrous crystals (1.69 g, 49%). mp 57 – 59 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.67 – 8.61 (m,
1-(Thiophen-2-yl)vin-2-yl cyclopropyl ketone (3dd) Synthesized according to GS1 from 2-thiophenecarboxaldehyde and methyl cyclopropyl ketone. Purified by column chromatography to yield a pale-yellow liquid that solidifies upon standing at room temperature. A recrystallization from methanol/water yielded pale yellow plates (2.78 g, 78%). mp 51 – 52 °C. 

1H NMR (400 MHz, CDCl₃) δ 7.72 (d, J = 15.8 Hz, 1H), 7.38 (d, J = 5.1 Hz, 1H), 7.29 (d, J = 3.6 Hz, 1H), 7.06 (dd, J = 5.1, 3.6 Hz, 1H), 6.69 (d, J = 15.7 Hz, 1H), 2.17 (tt, J = 7.8, 4.5 Hz, 1H), 1.19 – 1.10 (m, 2H), 1.02 – 0.90 (m, 2H). 13C NMR (101 MHz, CDCl₃) δ 199.7, 140.2, 134.5, 131.7, 128.7, 128.4, 127.2, 125.3, 20.0, 11.4. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C₁₀H₁₁NO⁺ 179.0525; found: 179.0523.

2-(1-Methylpyrazol-4-yl)vinyl cyclopropyl ketone (3ee) Synthesized according to GS1 from 1-methylpyrazole-4-carboxaldehyde and methyl cyclopropyl ketone. White crystals (0.745 g, 85%). mp 80 – 81 °C. 1H NMR (400 MHz, CDCl₃) δ 7.72 (s, 1H), 7.57 (s, 1H), 7.48 (d, J = 16.0 Hz, 1H), 6.62 (d, J = 16.0 Hz, 1H), 3.91 (s, 3H), 2.14 (tt, J = 7.8, 4.5 Hz, 1H), 1.15 – 1.07 (m, 2H), 0.98 – 0.86 (m, 2H). 13C NMR (101 MHz, CDCl₃) δ 200.0, 139.0, 132.6, 130.7, 124.6, 118.8, 39.3, 19.6, 11.2. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C₁₀H₁₃N₂O⁺ 177.1022; found: 177.1023.

Styryl 1-(ethylcarboxy)cycloprop-1-yl ketone (3ff) 1-acetyl-1-carbethoxycyclopropane. Into DMF (300 mL) was dissolved ethyl acetoacetate (32.54 g, 250 mmol) and 1,2-dibromoethane (65.75 g, 350 mmol), and anhydrous potassium carbonate (127.8
g, 975 mmol) was added. The slurry was stirred at 60 °C for 24 hours, after which water (300 mL) was added. The produce was extracted with diethyl ether (200 mL x 3), and the combined organic layers were washed with water (200 mL x 2), then brine (200 mL), followed by drying over anhydrous magnesium sulfate. The solvent was removed under vacuum, and 1-acetyl-1-carbethoxycyclopropane was isolated by distillation under light vacuum as a free-flowing colorless liquid (29.3 g, 75%). $^1$H NMR (400 MHz, CDCl$_3$) δ 4.19 (q, J = 7.1 Hz, 2H), 2.45 (s, 3H), 1.44 (s, 4H), 1.27 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 203.2, 171.1, 61.3, 35.2, 30.0, 19.2, 14.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_8$H$_{13}$O$_3^+$ 157.0859; found: 157.0861. Styryl 1-(ethylcarboxy)cycloprop-1-yl ketone was synthesized according to GS1 using benzaldehyde and 1-acetyl-1-carbethoxycyclopropane. White micaceous crystals (.471 g, 39%). mp 115 – 117 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.92 (d, J = 15.4 Hz, 1H), 7.57 (m, 2H), 7.50 – 7.37 (m, 3H), 6.58 (d, J = 15.4 Hz, 1H), 2.05 (distorted q, J = 4.5 Hz, 2H), 1.85 (distorted q, J = 4.5 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 199.8, 172.6, 147.8, 133.7, 131.8, 129.3, 129.0, 117.3, 32.3, 21.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{15}$H$_{17}$O$_3^+$ 245.1172; found: 245.1170.

4-Methoxystyryl cyclopropyl ketone oxime (4) 3r (1.01 g, 5 mmol) was dissolved in ethanol (50 mL), and hydroxylamine hydrochloride (0.382 g, 1.1 mmol) and pyridine (0.474 g, 1.2 mmol) were added. The solution was heated to 60°C for 18 hours. The solution was cooled, and water (100 mL) was added, which formed light tan, fibrous crystals (0.671 g, 62%). mp 151 – 154 °C (darkens at 130 °C). $^1$H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 7.57 – 7.48 (m, 2H), 7.33 (d, J = 16.7 Hz, 1H), 7.20 (d, J = 16.7 Hz, 1H), 7.00 – 6.91 (m, 2H), 3.77 (s, 3H), 1.78 (tt, J = 8.2, 5.1 Hz, 1H), 0.78 – 0.71 (m, 2H), 0.71 – 0.64 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ
Ethyl 4-chloro-α-cyanocinnamate (5a) 4-chlorobenzaldehyde (3.51 g, 25 mmol) and ethyl cycanoacetate (3.11 g, 27.5 mmol) were dissolved in ethanol, and pyrrolidine (0.355 g, 2 mmol) was added to the solution, which quickly turned yellow. Within ten minutes, white needles precipitated from the solution. After sitting at 22 °C for 2 hours, water (50 mL) was added, and the solid was filtered off and washed with water. The white crystalline solid was dried, yielding white crystals (5.38, 91%). mp 91 – 93 ºC. ¹H NMR (400 MHz, CDCl₃) δ 8.20 (s, 1H), 7.97 – 7.89 (m, 2H), 7.52 – 7.44 (m, 2H), 4.38 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 162.4, 153.6, 139.7, 132.3, 130.0, 129.8, 115.4, 103.6, 63.0, 14.3. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C₁₂H₁₁ClNO₂⁺ 236.0473; found: 236.0474.

Ethyl 4-methoxy-α-cyanocinnamate (5b) Following the same procedure as for 5a, but using p-anisaldehyde (3.40 g, 25 mmol) instead of 4-chlorobenzaldehyde. Light yellow needles (5.72 g, 99%). mp 82 – 83 ºC. ¹H NMR (400 MHz, CDCl₃) δ 8.14 (s, 1H), 8.01 – 7.94 (m, 2H), 7.01 – 6.93 (m, 2H), 4.34 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 163.8, 163.2, 154.5, 133.7, 124.4, 116.3, 114.8, 99.3, 62.5, 55.7, 14.3. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C₁₃H₁₄NO₃⁺ 232.0968; found: 232.0971.

General synthesis of methyl styryl sulfides (GS2): Diethyl (methylthiomethyl)-phosphonate (5 mmol, 0.99 g) was dissolved in anhydrous THF (25 mL) in a 100 mL round-bottom flask under argon. The solution was cooled to -78 ºC, and n-butyllithium (nominally 2.5 M in hexane, 5.5 mmol) was added dropwise over 5 minutes, and the reaction was stirred at -78 ºC for 15 minutes, and then at 22 ºC for 1 hour. The reaction was then cooled to -78 ºC again, and then an aldehyde (7.5 mmol) dissolved in THF (2 mL) was added over 5 minutes. The
reaction was stirred at -78 ºC for 15 minutes, and then brought to 22 ºC over 30 minutes, and then stirred at 50 ºC for 12 hours. The reaction was then quenched by the addition of water (25 mL) and hexane (25 mL). The two layers were separated, and the aqueous layer was extracted once more with hexane (25 mL). The combined organic layers were then washed with water (20 mL), 1 M hydrochloric acid (20 mL), 1 M sodium hydroxide (20 mL), and brine (20 mL), then dried over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the crude styryl sulfide was purified by column chromatography on silica using a gradient of 99:1 to 90:1 hexane:ethyl acetate.

**4-Chlorostyryl methyl sulfide (6a)** From 4-chlorobenzaldehyde. White plates after recrystallization from hexane. (618 mg, 67%). mp 55 – 56 ºC. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.28 – 7.19 (m, 4H), 6.78 (d, $J = 15.4$ Hz, 1H), 6.25 (d, $J = 15.5$ Hz, 1H), 2.38 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 135.8, 132.3, 128.9, 126.9, 126.7, 123.5, 14.9. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_9$H$_{10}$ClS$^+$ 185.0186; found: 185.0188.

**4-(Trifluoromethyl)styryl methyl sulfide (6b)** From 4-(trifluoromethyl)benzaldehyde. White plates after recrystallization from hexane. (705 mg, 65%). mp 54 – 55 ºC. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.56 – 7.50 (m, 2H), 7.40 – 7.34 (m, 2H), 6.94 (d, $J = 15.4$ Hz, 1H), 6.30 (d, $J = 15.5$ Hz, 1H), 2.41 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 140.5 (q, $J = 1.5$ Hz), 129.2, 128.3 (q, $J = 32.4$ Hz), 125.4, 124 (q, $J = 272.5$ Hz), 122.7. $^{19}$F NMR (377 MHz, CDCl$_3$) $\delta$ -62.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{10}$H$_{10}$F$_3$S$^+$ 219.0450; found: 219.0449.

**4-Methoxystyryl methyl sulfide (6c)** From 4-anisaldehyde. White solid (0.554 g, 61%). mp 71 – 73 ºC. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.26 – 7.20 (m, 2H), 6.88 – 6.80 (m, 2H), 6.62 (d, $J = 15.4$ Hz, 1H), 6.30 (d, $J = 15.4$ Hz, 1H), 3.80 (s, 3H), 2.37 (s, 3H). $^{13}$C NMR (101 MHz,
CDCl$_3$ $\delta$ 158.7, 130.2, 126.7, 125.0, 123.3, 114.2, 55.4, 15.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{10}$H$_{13}$OS$^+$ 181.0682; found: 181.0681.

**4-Methoxystyryl methyl sulfoxide (7)** In a 25 mL round-bottom flask, sodium periodate (214 mg, 1 mmol) was dissolved in water (10 mL), and the solution was cooled to 0 ºC. 6c (180 mg, 1 mmol) was dissolved in acetonitrile (5 mL) and this solution was added to the sodium periodate solution in one portion. The slurry was stirred overnight at 0 ºC, and the product was extracted three times with ethyl acetate (10 mL each). The combined organic extracts were washed with brine (20 mL), and the organic layer was dried over anhydrous magnesium sulfate. Removal of the solvent under vacuum produced a white solid that was recrystallized from 8:2 MTBE:hexane to give white flakes (182 mg, 93%). mp 73 – 74 ºC. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.45 – 7.38 (m, 2H), 7.19 (d, $J$ = 15.5 Hz, 1H), 6.94 – 6.87 (m, 2H), 6.76 (d, $J$ = 15.5 Hz, 1H), 3.83 (s, 3H), 2.69 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 161.1, 136.7, 129.7, 129.3, 126.5, 114.5, 55.5, 41.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{10}$H$_{13}$O$_2$S$^+$ 197.0631; found: 197.0634.

**Ethyl 3-(4-chlorophenyl)-3-oxopropanedithioate (8a) and 1-(4-chlorophenyl)-3,3-bis(ethylthio)prop-2-en-1-one (8b)** 4-chloroacetophenone (7.73 g, 50 mmol), carbon disulfide (5.71 g, 75 mmol), and ethyl bromide (13.62 g, 125 mmol) were added to THF (100 mL), and the solution was cooled to 0 ºC. Sodium hydride (60% dispersion in mineral oil, 4.40 g NaH, 110 mmol) was added portion-wise, and the solution turned deep yellow-orange. The reaction was stirred at this temperature for 1 hour, before warming and adding ice (100 g) to quench remaining sodium hydride. Approximately 50 mL of THF was removed under vacuum, and the oily mixture was extracted with 2:1 hexane:ethyl acetate (100 mL x 2). The combined organic layers were washed with water (100 mL) and then brine (100 mL), before drying over anhydrous
magnesium sulfate. The solvent was removed under vacuum, and the crude product was purified by column chromatography on approximately 200 g of silica using an eluent gradient of 1:9 to 1:3 ethyl acetate:hexane. The ketene dithioacetal (8b) elutes before 8a. **8a:** Yellow solid (1.21 g, 9.4%). mp 58 – 60 ºC. ¹H NMR (400 MHz, CDCl₃) δ 15.10 (s, 1H), 7.84 – 7.76 (m, 2H), 7.47 – 7.38 (m, 2H), 6.84 (s, 1H), 3.28 (q, J = 7.4 Hz, 2H), 1.38 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 217.0, 168.2, 138.1, 132.9, 129.2, 128.0, 107.8, 28.1, 13.0. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C₁₁H₁₂ClO₅S₂⁺ 259.0013; found: 259.0010. **8b:** Recrystallized from hexane. Yellow acicular solid (9.40 g, 66%). mp 82 – 83 ºC. ¹H NMR (400 MHz, CDCl₃) δ 7.86 – 7.78 (m, 2H), 7.42 – 7.34 (m, 2H), 6.73 (s, 1H), 3.07 (q, J = 7.4 Hz, 2H), 3.05 (q, J = 7.4 Hz, 2H), 1.42 (t, J = 7.4 Hz, 3H), 1.36 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 184.3, 166.1, 138.0, 137.8, 129.2, 128.8, 109.5, 28.5, 25.8, 13.9, 12.6. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C₁₃H₁₆ClO₅S₂⁺ 287.0326; found: 287.0329.

**3-(4-Chlorophenyl)-5-(ethylthio)isothiazole (9)** In a modification of a known procedure, ²¹ 8a (518 mg, 2 mmol) was dissolved in acetic acid (10 mL) in a 50 mL conical flask open to air. Ammonium acetate (771 mg, 5 mmol) was added to the solution, and the reaction was heated to 120 ºC for 24 hours, during which time the solution turns bright red. Water (20 mL) was added, and the product was extracted twice using diethyl ether (20 mL x 2). The combined organic layers were washed with water (20 mL x 2), then sodium bicarbonate (25 mL), and then brine (25 mL), and the red solution was then dried over anhydrous magnesium sulfate. The solvent was then removed to yield a deep red solid. Column chromatography was performed using 15% diethyl ether in hexane to yield the title compound as a red solid (372 mg, 73%). The red impurity could be removed by careful recrystallization from hexane to yield colorless prisms; however, NMR analysis suggested that the unknown red impurity comprised less than 2% of the
column-purified product. mp 58 – 59 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.88 – 7.80 (m, 2H), 7.43 – 7.38 (m, 2H), 7.37 (s, 1H), 7.36 (q, $J = 7.4$ Hz, 2H), 7.35 (q, $J = 7.4$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 166.7, 163.1, 135.4, 133.2, 129.1, 128.2, 120.9, 30.8, 14.7. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{11}$ClNS$_2^+$ 256.0016; found: 256.0016.

3-(4-Chlorophenyl)-5-(ethylthio)isoxazole (10) In a modification of a known procedure,$^{22}$ a 100 mL round-bottom flask, sodium (0.690 g, 30 mmol) was added to methanol (50 mL) at 0 °C to form a solution of sodium methoxide. After the complete reaction of sodium, hydroxylamine hydrochloride (1.39 g, 20 mmol) was added and stirred for 15 minutes, followed by the addition of $^8b$ (1.43 g, 5 mmol). The reaction was then refluxed for 16 hours, followed by cooling to room temperature. The methanol was removed under vacuum, and the residue was redissolved in ethyl acetate and water. The organic layer was washed with water (20 mL), 1 M hydrochloric acid (20 mL), and brine (20 mL), and then dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the product was purified by column chromatography. White crystals (144 mg, 12%). mp 65 – 66 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.74 – 7.68 (m, 2H), 7.46 – 7.40 (m, 2H), 6.43 (s, 1H), 3.10 (q, $J = 7.4$ Hz, 2H), 1.42 (t, $J = 7.4$ Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 167.4, 162.2, 136.3, 129.4, 128.2, 127.5, 102.1, 27.9, 15.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{11}$ClNOS$_2^+$ 240.0244; found: 240.0242.

5(3)-(4-Chlorophenyl)-3(5)-(ethylthio)pyrazole (11) In a modification of a known procedure,$^{23}$ $^8$b (1.43 g, 5 mmol) was dissolved in ethanol (25 mL), and hydrazine monohydrate (623 mg, 2.5 mmol) was added. The solution was heated to 70 °C for 16 hours, after which the yellow color of the starting material had disappeared. The ethanol was removed under vacuum, and the crude, thick liquid remaining was dissolved in ethyl acetate (25 mL), and washed with water (25 mL x 2), 1 M hydrochloric acid (20 mL), 0.5 M sodium hydroxide (20 mL), and brine
(20 mL), before drying over anhydrous magnesium sulfate. The product was isolated by recrystallization from boiling heptane to give the product as white plates (1.09 g, 92%). mp 97 – 99 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 11.0 (broad s, 1H), 7.66 – 7.58 (m, 2H), 7.38 – 7.30 (m, 2H), 6.59 (s, 1H), 2.87 (q, $J = 7.4$ Hz, 2H), 1.29 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 149.3, 139.6, 134.3, 130.1, 129.1, 127.0, 107.0, 30.0, 15.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{12}$ClN$_2$S$_2^+$ 239.0404; found: 239.0406.

4-Chloro-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (12a) Compound 11 (477 mg, 2 mmol) was dissolved in DMF (10 mL), and N-chlorosuccinimide (274 mg, 2.05 mmol) was added in one portion. The solution was heated to 70 ºC for 1 hour. The reaction was cooled, and water (25 mL) was added. The product was extracted with ethyl acetate, and the organic layer was washed twice with water (25 mL each) and brine (25 mL), followed by drying over anhydrous magnesium sulfate. The solvent was removed, and the product was purified by recrystallization from hexane to yield light pink botryoidal crystals (425 mg, 78%). mp 102 – 104 ºC. $^1$H NMR (600 MHz, CDCl$_3$) δ 11.3 (broad s, 1H), 7.71 (d, $J = 8.4$ Hz, 2H), 7.40 – 7.35 (m, 2H), 2.90 (q, $J = 7.4$ Hz, 2H), 1.27 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 144.3 (broad), 135.0, 129.5, 129.1, 128.4, 127.1, 112.7 (broad), 29.0, 15.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{12}$ClN$_2$S$_2^+$ 273.0015; found: 273.0017.

3-(4-Chlorophenyl)-5-(ethylthio)-4-iodopyrazole (12b) A procedure similar to that of 12a was followed at the 1-mmol scale, using N-iodosuccinimide (231 mg, 1.02 mmol) instead of NCS. White crystals (327 mg, 90%) after recrystallization from hot heptane. mp 113 – 115 ºC. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.9 (broad s, 1H), 7.69 – 7.64 (m, 2H), 7.44 – 7.38 (m, 2H), 2.97 (q, $J = 7.4$ Hz, 2H), 1.31 (t, $J = 7.4$ Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 149.1 (broad), 144.8
(broad), 135.3, 129.4, 129.2, 129.0, 67.2 (broad), 29.2, 15.1. HRMS (+ESI-QTOF) m/z: [M+H]⁺ calcd for C₁₁H₁₁ClIN₂S⁺ 364.9371; found: 364.9370.

3-(4-Chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a) Compound 8b (239 mg, 1 mmol) was dissolved in DMF (5 mL) and the solution was cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 1.2 mmol, 28.8 mg NaH) was added in a single portion, and the reaction was stirred for 15 min. Methyl iodide (156 mg, 1.1 mmol) was added in one portion, and the reaction was stirred at 0 °C for 30 minutes. The reaction was quenched by the addition of water (25 mL), and the aqueous suspension was extracted with ethyl acetate (25 mL). The organic layer was then washed with water (20 mL), 1 M hydrochloric acid (10 mL), 1 M sodium hydroxide (10 mL), and brine (20 mL), before drying the organic layer over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the crude product was purified by column chromatography. The crude product was a mixture of two isomers, easily discernable using TLC with 15:85 ethyl acetate:hexane. The title compound was isolated as a white solid (0.193 g, 76%, R_f = 0.6), and the minor isomer, 5-(4-chlorophenyl)-3-(ethylthio)-1-methylpyrazole (13a’), as a colorless liquid (35.4 mg, 14%, R_f = 0.35). Structural identity of isomers was confirmed using HMBC NMR. 13a: mp 60 – 61 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.74 – 7.66 (m, 2H), 7.41 – 7.31 (m, 2H), 6.60 (s, 1H), 3.95 (s, 3H), 2.81 (q, J = 7.3 Hz, 2H), 1.29 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 149.6, 135.7, 133.6, 131.8, 128.9, 126.7, 108.3, 36.9, 30.5, 15.0. HRMS (+ESI-QTOF) m/z: [M+H]⁺ calcd for C₁₂H₁₄ClIN₂S⁺ 253.0561; found: 253.0562. Minor isomer (13a’): ¹H NMR (600 MHz, CDCl₃) δ 7.46 – 7.40 (m, 2H), 7.36 – 7.31 (m, 2H), 6.28 (s, 1H), 3.83 (s, 3H), 2.96 (q, J = 7.3 Hz, 2H), 1.35 (t, J = 7.3 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 144.7, 144.0, 135.0, 130.1, 129.2, 128.7, 108.4, 37.7, 28.5, 15.2. HRMS (+ESI-QTOF) m/z: [M+H]⁺ calcd for C₁₂H₁₄ClIN₂S⁺ 253.0561; found: 253.0563.
1-(Carbethoxy)methyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13b) Following a procedure similar to that of 13a at the 1-mmol scale, methyl chloroacetate (119 mg, 1.1 mmol) was used instead of methyl iodide. Crude product was purified from hexane (5 mL) left at -20°C overnight to yield white crystals (282 mg, 91%). mp 55 – 56 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.73 – 7.68 (m, 2H), 7.38 – 7.33 (m, 2H), 6.70 (s, 1H), 5.09 (s, 2H), 3.78 (s, 3H), 2.80 (q, $J$ = 7.3 Hz, 2H), 1.29 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 168.4, 150.8, 136.6, 133.9, 131.5, 128.9, 127.0, 109.5, 52.8, 50.8, 30.9, 15.0. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{14}$H$_{16}$ClN$_2$O$_2$S $^+$ 311.0616; found: 311.0615.

1-Acetyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13c) Compound 11 (239 mg, 1 mmol) was dissolved in chloroform (10 mL), and pyridine (95 mg, 1.2 mmol) was added. The solution was cooled to 0 °C, and acetyl chloride (86 mg, 1.1 mmol) was added dropwise. The reaction was warmed to 22 °C, and stirred for 1 hour. The reaction was diluted with hexane (20 mL), and washed with water (20 mL x 2), then brine (20 mL), followed by drying over anhydrous magnesium sulfate. The solvent was removed, and the crude solid was recrystallized from hot heptane to yield white needles (0.257 g, 91%). mp 131 – 132 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.81 – 7.74 (m, 2H), 7.44 – 7.36 (m, 2H), 6.40 (s, 1H), 2.99 (q, $J$ = 7.4 Hz, 2H), 2.73 (s, 3H), 1.45 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.4, 153.5, 145.0, 135.3, 130.4, 129.1, 127.6, 103.5, 27.7, 22.9, 13.2. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{13}$H$_{14}$ClN$_2$O$_2$S $^+$ 281.0510; found: 281.0511.

1-Carbethoxy-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13d) Identical to the procedure of 13c, using ethyl chloroformate (119 mg, 1.1 eq), instead of acetyl chloride. Off-white prisms (0.303 g, 97%). mp 76 – 77 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.82 – 7.75 (m, 2H), 7.43 – 7.35 (m, 2H), 6.41 (s, 1H), 4.55 (q, $J$ = 7.1 Hz, 2H), 3.02 (q, $J$ = 7.4 Hz, 2H), 1.49 (t,
J = 7.2 Hz, 3H), 1.45 (t, J = 7.4 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 154.1, 150.5, 146.1, 135.3, 130.2, 129.0, 127.9, 103.7, 65.0, 27.9, 14.4, 13.3. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C14H16ClN2O2S+ 311.0616; found: 311.0617.

5(3)-(4-Chlorophenyl)-3(5)-(ethylsulfinyl)pyrazole (14) In a 25 mL round-bottom flask, sodium periodate (214 mg, 1 mmol) was dissolved in water (5 mL), and the solution was cooled to 0 ºC. 11 (239 mg, 1 mmol) was dissolved in acetonitrile (5 mL) and this solution was added to the sodium periodate solution in one portion. The slurry was stirred overnight at 0 ºC, and the product was extracted three times with ethyl acetate (10 mL each). The combined organic extracts were washed with water (20 mL) and then brine (20 mL), and the organic layer was dried over anhydrous magnesium sulfate. The solvent was removed, and the crude product was purified by recrystallization from hot heptane to yield white flakes (0.236 g, 93%). mp 141 – 143 ºC. 1H NMR (400 MHz, CDCl3) δ 10.74 (broad s, 1H), 7.64 – 7.56 (m, 2H), 7.41 – 7.33 (m, 2H), 6.93 (s, 1H), 3.22 – 3.09 (m, 2H), 1.29 (t, J = 7.4 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 152.4, 145.7, 135.1, 129.5, 127.5, 127.2, 102.3, 48.7, 6.6. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C11H12ClN2O+ 255.0353; found: 255.0354.

3-Phenylisoxazol-5-one (15) In a modification of a literature procedure,24 hydroxylamine hydrochloride (5.21 g, 75 mmol) and sodium acetate (6.15 g, 75 mmol) were dissolved in ethanol (100 mL), and the solution was stirred for 10 minutes. Ethyl benzoylacetaete (9.61 g, 50 mmol) was added, and the solution was refluxed for 8 hours. Ethanol was removed under vacuum, and the crude material was dissolved in water (50 mL) and ethyl acetate (50 mL). The separated organic layer was dried over anhydrous magnesium sulfate, and the crude material was recrystallized from hexane/ethyl acetate to yield white crystals (6.18 g, 77%). mp 153 – 155 ºC. 1H NMR (600 MHz, CDCl3) δ 7.71 – 7.66 (m, 2H), 7.57 – 7.51 (m, 1H), 7.50 – 7.46 (m, 2H),
3.81 (s, 2H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 174.8, 163.2, 132.3, 129.4, 127.8, 126.7, 34.2. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{9}$H$_{8}$NO$_2^+$ 162.0550; found: 162.0547.

3-Phenyl-5-chloroisoxazole (16) In a modification a literature procedure,$^{25}$ 15 (3.22 g, 20 mmol) was dissolved in phosphorus oxychloride (100 mmol, 15.3 g), and the solution was cooled to 0 °C, at which point triethylamine (2.02 g, 20 mmol) was added dropwise over 1 minute. The reaction was then refluxed for 6 hours, and the reaction was then quenched by the careful addition of ice (100 g), and the pH was adjusted to approximately 8 by the addition of 6 M sodium hydroxide. The product was then extracted with ethyl acetate (50 mL x 2), and the combined organic extracts were washed with water (50 mL) and brine (50 mL), and then dried over anhydrous magnesium sulfate. The crude product was purified by recrystallization from hexane/ethyl acetate to yield pale tan crystals (2.39 g, 67%). mp 46 – 47 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.79 – 7.72 (m, 2H), 7.51 – 7.43 (m, 3H), 6.48 (s, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 164.3, 155.2, 130.7, 129.2, 128.3, 126.8, 99.7. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{9}$H$_{7}$ClNO$_2^+$ 180.0211; found: 180.0212.

SCN hatching assays

Field soil, collected from Muscatine, IA, USA, and naturally infested with SCN (HG type 2),$^{26}$ was used to grow susceptible soybeans (cv. Williams 82). Females of SCN were collected on a 250-µm-pore sieve nested under an 850-µm-pore sieve after dislodgement from roots of 30- to 60-day-old plants under a stream of tap water. Collected females were isolated from root fragments and soil debris by sucrose centrifugation using a 4 M sucrose solution, and the females were rinsed well with tap water.$^{27}$ Females were crushed with a motorized rubber stopper to release the eggs, which were collected on a 25-µm-pore sieve under a 75-µm-pore sieve.$^{28}$ The eggs were separated from remaining debris by sucrose centrifugation using 454 g/L of sucrose,$^{27}$ followed by three rinses with autoclaved deionized water prior to use the hatching assays.
Stock solutions of nematicides were made by dissolving 10.0 – 20.0 mg of a compound in a solution of 10% TritonX-100 in ethanol in a 1 mL volumetric flask; the nominal concentration was determined based on the actual mass added to the flask. This stock solution was then used to make 100 mL of a 100 ppm solution of the compound in deionized water, and 10% TritonX-100 in ethanol was added to ensure each solution had a final concentration of 0.1% TritonX-100 and 0.9% ethanol. Solutions were stored in tightly-sealed contained in the dark.

As a screening method, SCN were continuously exposed to these 100 ppm solutions over 15 days, using autoclaved deionized water, 5.5 mM zinc sulfate, and a 0.1%/0.9% TritonX-100/ethanol solvent black as three different controls. Because zinc sulfate stimulates in vitro hatching, it provides a useful reference point for any hatch-stimulating compounds by serving as a positive control.

In each assay, sieves were constructed from plastic test tube caps (2 cm diameter, 1.5 cm height) with the end moved, and a 30 μm pore nylon mesh (Elko Filtering Co.) affixed in its place. Approximately 300 SCN eggs were dispensed into each sieve, and these loaded sieves were each placed in a well of individual sterile polystyrene 6-well microplate (Greiner Bio-One North America), and 3 mL of the treatment or control solution was added to the well containing the sieve. The microplates were covered with lids, and incubated at 25 ºC in the dark. After three days, the sieves were moved to the next empty well in the microplate, and 3 mL of fresh solution was added to the well, reserving the old solution in its well. After transferal of the sieve, the number of hatched juveniles that migrated through the sieve into the well were counted. This process of transferring the sieve to new solution was repeated on days 6, 9 and 12. After day 15, a final counting of hatched juveniles in the last well was performed, and the remaining unhatched
eggs were also counted. The total number of hatched second-stage juveniles in the assay was determined as the sum of counted juveniles in the trial at days 3, 6, 9, 12, and 15.

**C. elegans screening assay and data analysis**

The fluorescence-based *C. elegans* screening assay has been described in detail previously (see Chapter 5 of this dissertation). Briefly, approximately 500 freshly-synchronized *C. elegans* were placed into the wells of a 96-well microplate containing 200 μL of M9 buffer and the compounds of interest ranging in concentration from 128 ppm down to 0.25 ppm in twofold dilutions. The nematode eggs were kept in the solution for four hours at 20 ºC, after which 20 μL of fluorescein diacetate solution (20 μg mL⁻¹ in M9 buffer) was added. The *C. elegans* were incubated for an additional 20 hours, after which the fluorescence of each well was determined on a Synergy HTX plate reader (Biotek®) using excitation and emission filters with 20 nm bandwidths (λ_ex: 485 nm; λ_em: 528 nm). Dose-response curves were fit to the fluorescence data with Cedargreen-Ritz-Streibig (CRS) hormesis models using the R programming language and the *drc* package; these models were then used to estimate 95% confidence intervals for EC₅₀ and HC₁₀ values. Statistical differences between treatments were determined using non-overlap of 95% confidence intervals.

**SCN experimental design and data analysis**

Because results from earlier trials were used to inform the synthesis of new compounds for future trials, the SCN studies were performed using an unbalanced incomplete block design, with deionized water, solvent blank (0.1% TritonX-100 and 0.9% ethanol in deionized water), and 5.5 mM zinc sulfate serving as controls in each block. Within each block, four technical replicates were performed for each treatment. For each replicate, the hatch percentage was logit-transformed; in cases where either none or all of the eggs had hatched, Hanley’s rule of three was used to estimate an upper or lower confidence bound, respectively, that was used for the logit
transformation. The logit-transformed hatch rates were then used to perform a linear regression with the treatment and block as fixed effects. Contrasts were used to compare treatments to the solvent control using the logit-transformed estimated marginal means of the treatments with Dunnett’s correction for multiple comparisons to obtain simultaneous 95% confidence intervals, which were then back-transformed from the logit scale.\textsuperscript{35}

**Results and Discussion**

Our first step in developing novel cinnamaldehyde analogs as nematicides was to screen compounds that were already commercially available. Cinnamaldehyde (1a) and the six close derivatives 1b-g were chosen for the initial screening. Additionally, β-nitrostyrene (2b), which is similar to cinnamaldehyde in that it contains a strong electron-withdrawing group off of a styrene scaffold, as well as several commercially-available β-nitrostyrene derivatives (2b-f), were added to the initial screen; the structures of these compounds are shown in Figure 6.1.

Table 6.1 summarizes the results of these treatments against SCN hatch rate inhibition at 100 ppm over 15 days, and the estimated dose that results in a loss of 50% of esterase activity in *C. elegans* compared to the baseline (EC\textsubscript{50}), which is likely an overestimation of the concentration needed to kill 50% of the nematode (LC\textsubscript{50}) due the effects of hormesis. Of the cinnamaldehydes and nitrostyrenes tested, only 4- fluorocinnamaldehyde (1b) and 4-hydroxy-3-methoxycinnamaldehyde (1e) did not induce a significant reduction in SCN hatch rates. 4-Methoxycinnamaldehyde (1d) has been previously reported to produce a hormesis-type effect in the fluorescein diacetate *C. elegans* bioassay, wherein sublethal concentrations of a potential toxicant causes greater fluorescence than the baseline level. The concentration at which a 10% increase in fluorescence over the baseline level is denoted as HC\textsubscript{10}, if it exists. Of all the cinnamaldehydes and nitrostyrenes tested, only 1d had an HC\textsubscript{10}. 
Because cinnamaldehyde and its derivatives are easily oxidized to cinnamic acids in the presence of oxygen, we next decided to explore styryl ketones as analogs of cinnamaldehyde. The simplest of these compounds, methyl styryl ketone (3a), is commercially available. We also synthesized 31 additional styryl ketones (3b-ff) via the aldol reaction using the appropriate aryl

![Figure 6.1. Cinnamaldehydes (1a-g) and β-nitrostyrenes (2a-f) screened as potential nematicides against SCN and *C. elegans*.](image_url)

| Compound | Hatch inhibition$^a$ | p-value$^b$ | EC$^{50}$ | HC$^{10}$ $^d$
|----------|---------------------|--------|---------|------
| 1a       | -98.9 [-99.5, -97.6] | <0.0001 **** | 367 [317, 425] |
| 1b       | -54.1 [-76.7, -15.5] | 0.01077 * | 307 [286, 330] |
| 1c       | -86 [-90.3, -80.1] | <0.0001 **** | 280 [250, 314] |
| 1d       | -87.3 [-91.6, -80.8] | <0.0001 **** | 240 [223, 259] | 5.96 [4.95, 7.18] |
| 1e       | -25.9 [-51.8, 8.5] | - | NT | NT |
| 1f       | -88 [-94.2, -75.6] | <0.0001 **** | 267 [235, 304] |
| 1g       | -88.1 [-92.2, -81.9] | <0.0001 **** | NT | NT |
| 2a       | -79.4 [-86.2, -69.6] | <0.0001 **** | 18.3 [13.4, 25.0] |
| 2b       | -58.6 [-74, -36.1] | <0.0001 **** | NT | NT |
| 2c       | -82.7 [-88.6, -74.1] | <0.0001 **** | NT | NT |
| 2d       | -88.8 [-93.2, -81.7] | <0.0001 **** | 81.9 [67.1, 100] |
| 2e       | -82.5 [-88.5, -73.8] | <0.0001 **** | 95.3 [74.5, 122] |
| 2f       | -77.5 [-86.2, -64] | <0.0001 **** | 23.9 [21.4, 26.7] |

$^a$Inhibition of hatching of SCN over 15 days of continuous exposure to 100 ppm of a compound compared to a solvent control. Values in brackets represent the 95% confidence interval. $^b$p-value statistical difference from the solvent control (**** <0.0001, *** <0.001, ** <0.01, * <0.05, - >0.1). $^c$Calculated EC$^{50}$ of the treatment against *C. elegans* in μM. $^d$Calculated HC$^{10}$ of the treatment against *C. elegans* in μM, when it exists. NT: not tested.
carboxaldehyde and methyl ketone derivatives as shown in Scheme 6.1; the structures of these compounds can be found in Figure 6.2. The styryl ketones are mostly cyclopropyl ketones, with a wide variety of electron-donating and electron-withdrawing substituents on the aromatic ring.
In addition to this array of ketones, several other cinnamaldehyde analogs were synthesized, including the oxime 4, ethyl α-cyanocinnamates 5a and 5b, methyl styryl sulfides 6a-6c, and the methyl styryl sulfoxide 7.

Table 6.2. contains the hatch inhibition rates of these cinnamaldehyde analogs against SCN, as well as the corresponding EC$_{50}$ and HC$_{10}$ concentrations for C. elegans, when applicable. We were somewhat surprised to find that there is not an obvious trend between the efficacy of the styryl ketones 3 against either SCN or C. elegans and the electron-donating or electron-withdrawing capacity of the substituents on the styryl ketone. Even when focusing on the subset of cyclopropyl ketones with small substituents in the para-position (specifically 3f, 3h-j, 3o-t, and 3y-aa), there is no clear correlation between the SCN hatch inhibition or C. elegans EC$_{50}$ and the Hammett parameter $\sigma_p$, used as a numerical estimate of the electronic effects of the substituent (Figure 6.3).

Of the styryl ketones tested, nearly three-fourths of them caused statistically significant reductions in SCN hatch over the 15-day exposure study. Numerically, amongst the cyclopropyl styryl ketones, the parent cyclopropyl ketone 3f, the 4-chloro derivative 3i, 4-bromo-2-fluorostyryl cyclopropyl ketone (3m), the 4-(trifluoromethyl) derivative (3o), and the 4-
Table 6.2. Efficacy data of compounds 3-7 against SCN hatching and *C. elegans* EC<sub>50</sub> and HC<sub>10</sub>. See Table 6.1 for table footnotes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hatch inhibition&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>HC&lt;sub&gt;10&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>-93.7 [-95.9, -90.4]</td>
<td>&lt;0.0001 ****</td>
<td>461 [424, 500]</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>-28.9 [-61.7, 20.2]</td>
<td>-</td>
<td>391 [376, 405]</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>-33.4 [-50.9, -11.7]</td>
<td>0.00325 **</td>
<td>270 [248, 294]</td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>-18.3 [-41.3, 10]</td>
<td>-</td>
<td>&gt;575</td>
<td></td>
</tr>
<tr>
<td>3e</td>
<td>-24.8 [-44.2, -1.1]</td>
<td>0.04662 *</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3f</td>
<td>-81 [-87.5, -71.5]</td>
<td>&lt;0.0001 ****</td>
<td>89.7 [61.5, 131]</td>
<td></td>
</tr>
<tr>
<td>3g</td>
<td>-49.2 [-67.7, -22.8]</td>
<td>0.00115 **</td>
<td>907 [784, 1050]</td>
<td></td>
</tr>
<tr>
<td>3h</td>
<td>-59.7 [-72.5, -42.2]</td>
<td>&lt;0.0001 ****</td>
<td>615 [590, 640]</td>
<td>4.52 [2.73, 7.49]</td>
</tr>
<tr>
<td>3i</td>
<td>-82.7 [-87.4, -76.4]</td>
<td>&lt;0.0001 ****</td>
<td>338 [280, 409]</td>
<td></td>
</tr>
<tr>
<td>3j</td>
<td>-51.5 [-69.3, -25.9]</td>
<td>0.00057 ***</td>
<td>47.6 [45.3, 49.9]</td>
<td>9.36 [7.4, 11.8]</td>
</tr>
<tr>
<td>3k</td>
<td>6.0 [-27.3, 45.7]</td>
<td>-</td>
<td>&gt;531</td>
<td></td>
</tr>
<tr>
<td>3l</td>
<td>-50.9 [-69.9, -23.1]</td>
<td>0.0149 **</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3m</td>
<td>-76.3 [-84, -65.2]</td>
<td>&lt;0.0001 ****</td>
<td>90.7 [82.2, 100]</td>
<td></td>
</tr>
<tr>
<td>3n</td>
<td>-5.2 [-35.7, 32.4]</td>
<td>-</td>
<td>&gt;570</td>
<td></td>
</tr>
<tr>
<td>3o</td>
<td>-80.9 [-86.6, -73]</td>
<td>&lt;0.0001 ****</td>
<td>515 [415, 639]</td>
<td></td>
</tr>
<tr>
<td>3p</td>
<td>-45.6 [-62.1, -23.8]</td>
<td>0.00032 ***</td>
<td>&gt;589</td>
<td></td>
</tr>
<tr>
<td>3q</td>
<td>35.6 [7.9, 65.1]</td>
<td>0.01319 *</td>
<td>734 [676, 796]</td>
<td></td>
</tr>
<tr>
<td>3r</td>
<td>-66.1 [-79, -46.6]</td>
<td>&lt;0.0001 ****</td>
<td>239 [220, 260]</td>
<td></td>
</tr>
<tr>
<td>3s</td>
<td>-53.2 [-70.4, -28.4]</td>
<td>0.00030 ***</td>
<td>282 [260, 307]</td>
<td>1.07 [0.452, 2.51]</td>
</tr>
<tr>
<td>3t</td>
<td>-53.9 [-65.5, -39.1]</td>
<td>&lt;0.0001 ****</td>
<td>&gt;592</td>
<td>11.6 [0.831, 161]</td>
</tr>
<tr>
<td>3u</td>
<td>-8.3 [-37.9, 28.4]</td>
<td>-</td>
<td>&gt;551</td>
<td>3.53 [2.27, 5.48]</td>
</tr>
<tr>
<td>3v</td>
<td>-34.4 [-53.7, -9.7]</td>
<td>0.01019 *</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3w</td>
<td>-65.6 [-76.5, -50.3]</td>
<td>&lt;0.0001 ****</td>
<td>1100 [918, 1330]</td>
<td></td>
</tr>
<tr>
<td>3x</td>
<td>-32.2 [-52, -6.9]</td>
<td>0.01748 *</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3y</td>
<td>-70.2 [-78.8, -58.7]</td>
<td>&lt;0.0001 ****</td>
<td>78.9 [74.1, 84]</td>
<td>0.569 [0.431, 0.752]</td>
</tr>
<tr>
<td>3z</td>
<td>-13.1 [-37.2, 16.2]</td>
<td>-</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3aa</td>
<td>-51.5 [-68.3, -27.8]</td>
<td>0.00025 ***</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3bb</td>
<td>-48.1 [-66.8, -21.6]</td>
<td>0.00146 **</td>
<td>581 [548, 617]</td>
<td></td>
</tr>
<tr>
<td>3cc</td>
<td>-35.8 [-54.8, -11.3]</td>
<td>0.00748 **</td>
<td>&gt;739</td>
<td></td>
</tr>
<tr>
<td>3dd</td>
<td>-69.5 [-81.2, -51.7]</td>
<td>&lt;0.0001 ****</td>
<td>345 [326, 366]</td>
<td></td>
</tr>
<tr>
<td>3ee</td>
<td>-14.2 [-38, 14.8]</td>
<td>-</td>
<td>&gt;726</td>
<td></td>
</tr>
<tr>
<td>3ff</td>
<td>-1.3 [-43.9, 55]</td>
<td>-</td>
<td>642 [558, 738]</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.7 [-20.7, 91.7]</td>
<td>-</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>-78.2 [-86.6, -65]</td>
<td>&lt;0.0001 ****</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>-2.4 [-44.6, 53.7]</td>
<td>-</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>-65.9 [-78.7, -46.9]</td>
<td>&lt;0.0001 ****</td>
<td>282 [266, 300]</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>-66 [-78.7, -47]</td>
<td>&lt;0.0001 ****</td>
<td>194 [173, 218]</td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td>-46.6 [-63.2, -24.5]</td>
<td>0.00032 ***</td>
<td>&gt;710</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-34 [-64.1, 11.5]</td>
<td>-</td>
<td>&gt;625</td>
<td></td>
</tr>
</tbody>
</table>
methylthio analog (3y) were the most potent inhibitors. Interestingly, the cyano derivative 3q promoted SCN egg attach over that of the blank control rate, an effect that was repeated in each of the four replicates performed for this compound. Although not as potent an SCN egg hatch promotor as zinc sulfate, this effect of 3q is of interest because hatch promotors could be used to induce hatching of SCN eggs when a non-host crop is planted, which would then reduce the SCN egg burden in the soil for a subsequent soybean crop.

The analogs 4-7 were developed to analyze the effects of replacing the ketone found in compounds in 3a-ff with other groups. The oxime 4, which is a direct analog of 3s, was far less effective in modulating SCN hatch rates. The 4-chloro-α-cyanocinnamate 5a was a significant inhibitor of hatching, while the 4-methoxy analog 5b had no significant effect. The styryl sulfide analogs 6a-c were synthesized to explore the effect of replacing the carbonyl with a sulfur atom; all three of these sulfides were significant hatch inhibitors against SCN; the methoxy analog 6c was not very effective against *C. elegans* compared to the 4-chloro and 4-trifluoromethyl analogs (6a and 6b, respectively). Additionally, 6c was more effective against SCN hatching than the sulfoxide 7, an oxidation product of 6c.

We then opted to synthesize a series of analogs of cinnamaldehyde containing an azole ring in place of the α,β-unsaturated carbonyl, with many of these compounds also possessing an alkylthio group to mimic the efficacy of the styryl sulfides 6a-c; additionally, these compounds were of interest to us because the biological activity of these alkylthio azoles has been less surveyed than other classes of compounds. A series of ten 3-(4-chlorophenyl)-5-ethylthio-azoles were synthesized starting from 4-chloroacetophenone as shown in Scheme 6.2. This series contained an isothiazole (9), and isoxazole (10), as well as eight pyrazoles (11-14), with the final structures as shown in Figure 6.4. In addition to the ten aforementioned azoles, 3-
The results of the azoles against SCN and *C. elegans* are shown in Table 6.3. Both the isothiazole 9 and isoxazole 10 were statistically significant inhibitors of SCN egg hatch. Additionally, 9 was the most potent compound against *C. elegans* in terms of EC$_{50}$, with a value of approximately 10.3 μM. Furthermore, the HC$_{10}$ of this compound against *C. elegans* was well below 1 μM, suggesting that 9 is capable of inducing an effect in nematodes at a very low concentration. The pyrazole 11 was not a statistically significant inhibitor of SCN egg hatch at 100 ppm, but the EC$_{50}$ of this compound was approximately 108 μM, which is on the order of magnitude of the best styryl ketones. The HC$_{10}$ of both of these compounds were remarkably low—below the lowest concentration used for screening compounds in the bioassay—and extrapolation suggests values well below 500 nM (Table 6.3). Additionally, both 9 and 11 produced very strong hormetic effects in *C. elegans*, with maximal fluorescence in the bioassay at levels twice that seen in the blank control (Figure 6.5). In comparison to 9 and 11, the potency of many of the other azoles was less remarkable. 13d, a carbamate-protected analog of 11, was a statistically significant inhibitor of SCN egg hatch; however, other *N*-substituted pyrazoles (13a-c) did not significantly reduce the SCN hatch rates; moreover, 13a-c were less effective against *C. elegans* compared to 11 both in terms of EC$_{50}$ and HC$_{10}$. It is worth noting that some substituted azoles have been previously explored for their nematicidal properties.$^{36-38}$

The overall correlation between the hatch modulation in SCN and the EC$_{50}$ values obtained from the *C. elegans* egg hatch bioassay is poor; there is no clear trend between these two bioassay end points (see Figure 6.6). However, there is some predictive power in using the rapid *C. elegans* bioassay as a predictive screen for determining which compounds may be active in the SCN bioassay. There were 33 compounds that produced estimable EC50 values against *C.
elegans (CE+ compounds); 30 of these compounds caused statistically significant modulation of SCN hatch rates (SCN+) compounds. Additionally, of the 11 compounds that did not lead to estimable EC50 values (CE-), seven did not significantly change the SCN hatch rate (SCN-).

Figure 6.6 graphically shows the four possible classes of these compounds, wherein classes 1 and
2 represent an agreement in bioassay results between *C. elegans* and SCN, while classes 3 and 4 contain compounds that were significant in only one of the bioassays. While the SCN hatch inhibition assay takes place over 15 days, the *C. elegans* bioassay can deliver results in 24 hours with a sensitivity of 91% and selectivity of 64%—although this sensitivity is lower than desirable, the compounds in class 3 (SCN+, CE-) were numerically less potent hatch inhibitors against SCN than many of those in class 1. *C. elegans* may therefore be useful as a rapid binary

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hatch inhibition</th>
<th>p-value</th>
<th>EC(_{50})</th>
<th>HC(_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>-71.2 [-80.7, -57.7]</td>
<td>&lt;0.0001 ****</td>
<td>12.9 [10.7, 15.4]</td>
<td>0.201 [0.063 0.644]</td>
</tr>
<tr>
<td>10</td>
<td>-45.1 [-62.6, -21.7]</td>
<td>0.00083 ***</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>-25 [-47.7, 3.7]</td>
<td>0.09239</td>
<td>115 [95.5, 139]</td>
<td>0.060 [0.011, 0.327]</td>
</tr>
<tr>
<td>12a</td>
<td>-36.3 [-65.6, 8.6]</td>
<td>-</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>12b</td>
<td>-8.9 [-52.9, 53.3]</td>
<td>-</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>13a</td>
<td>-13.4 [-44.5, 25.8]</td>
<td>-</td>
<td>333 [244, 456]</td>
<td>1.43 [0.692, 2.94]</td>
</tr>
<tr>
<td>13b</td>
<td>-10.3 [-49.6, 43.6]</td>
<td>-</td>
<td>166 [152, 181]</td>
<td>2.02 [1.03, 3.99]</td>
</tr>
<tr>
<td>13c</td>
<td>-16.4 [-44.7, 19.9]</td>
<td>-</td>
<td>&gt;456</td>
<td></td>
</tr>
<tr>
<td>13d</td>
<td>-60.5 [-76.1, -37]</td>
<td>&lt;0.0001 ****</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-22.3 [-49, 12.6]</td>
<td>-</td>
<td>&gt;502</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>-38.2 [-66.9, 6.6]</td>
<td>0.09031</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>-60.8 [-79.9, -28]</td>
<td>0.00179 **</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.5. Dose-response curves with significant hormetic effects for pyrazole 9 (plot a) and isothiazole 11 (plot b) against *C. elegans*.
predictor of SCN hatch modulation, thereby reducing the number of compounds that need to be screened in a time-consuming bioassay. Additionally, this low correlation suggests that it may be possible to selectively target the harmful plant-parasitic nematodes while leaving the population of other soil-dwelling nematodes intact.

**Conclusion**

By screening more than 60 analogs of cinnamaldehyde against *Heterodera glycines*, we have discovered several potent inhibitors of SCN egg hatch, as well as a single styryl ketone that increases the SCN egg hatch compared to the solvent control. Unfortunately, there were no clear trends in the relationship between the SCN hatch modulation and the chemical structures of even closely-related styryl ketones. Two heterocyclic analogs of cinnamaldehyde, a pyrazole and an
isothiazole, were potent egg hatch inhibitors of both SCN and *C. elegans*, and induced physiological responses in *C. elegans* detectable at the sub-μM level using a fluorescence-based bioassay. Although the correlation between SCN egg hatch modulation and dose-response information in *C. elegans* was poor, the rapid bioassay using *C. elegans* provided some binary predictive power in determining which compounds would likely cause hatch modulation in SCN.

**Acknowledgements**

This project was funded by AMVAC Chemical Corporation under Iowa State University grant GR-015523-00001 and by the Iowa Agricultural Experimental Station. The authors would like to acknowledge Edmund J. Norris (University of Florida) for his assistance in securing funding that led to this project.

**References**


Appendix

4-methoxystyryl methyl ketone (3b) $^1$H NMR (400 MHz, CDCl$_3$)

4-methoxystyryl methyl ketone (3b) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-methoxy styryl isopropyl ketone (3c) $^1$H NMR (400 MHz, CDCl$_3$)

4-methoxy styryl isopropyl ketone (3c) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-chlorostyryl t-butyl ketone (3d) \(^1\)H NMR (400 MHz, CDCl\(_3\))

4-chlorostyryl t-butyl ketone (3d) \(^13\)C NMR (101 MHz, CDCl\(_3\))
4-bromostyryl t-butyl ketone (3e) $^1$H NMR (400 MHz, CDCl$_3$)

![1H NMR spectrum](image)

4-bromostyryl t-butyl ketone (3e) $^{13}$C NMR (101 MHz, CDCl$_3$)

![13C NMR spectrum](image)
styryl cyclopropyl ketone (3f) $^1$H NMR (400 MHz, CDCl$_3$)

styryl cyclopropyl ketone (3f) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-fluorostyryl cyclopropyl ketone (3g) $^1$H NMR (400 MHz, CDCl$_3$)

3-fluorostyryl cyclopropyl ketone (3g) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-fluorostyryl cyclopropyl ketone (3g) $^{19}$F NMR (376 MHz, CDCl$_3$)

4-fluorostyryl cyclopropyl ketone (3h) $^1$H NMR (400 MHz, CDCl$_3$)
4-fluorostyryl cyclopropyl ketone (3h) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-fluorostyryl cyclopropyl ketone (3h) $^{19}$F NMR (376 MHz, CDCl$_3$)
4-chlorostyryl cyclopropyl ketone (3i) $^1$H NMR (400 MHz, CDCl$_3$)

4-chlorostyryl cyclopropyl ketone (3i) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-bromostyryl cyclopropyl ketone (3j) $^1$H NMR (400 MHz, CDCl$_3$)

4-bromostyryl cyclopropyl ketone (3j) $^{13}$C NMR (101 MHz, CDCl$_3$)
2,4-dichlorostyryl cyclopropyl ketone (3k) $^1$H NMR (400 MHz, CDCl$_3$)

2,4-dichlorostyryl cyclopropyl ketone (3k) $^{13}$C NMR (101 MHz, CDCl$_3$)
3,4-dichlorostyryl cyclopropyl ketone (3l) $^1$H NMR (400 MHz, CDCl$_3$)

\[ \text{Chemical Structure Image} \]

3,4-dichlorostyryl cyclopropyl ketone (3l) $^{13}$C NMR (101 MHz, CDCl$_3$)

\[ \text{Chemical Structure Image} \]
4-bromo-2-fluorostyryl cyclopropyl ketone (3m) $^1$H NMR (400 MHz, CDCl$_3$)

4-bromo-2-fluorostyryl cyclopropyl ketone (3m) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-bromo-2-fluorostyryl cyclopropyl ketone (3m) $^{19}$F NMR (376 MHz, CDCl$_3$)

4-chloro-2-fluorostyryl cyclopropyl ketone (3n) $^1$H NMR (400 MHz, CDCl$_3$)
4-chloro-2-fluorostyryl cyclopropyl ketone (3n) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-chloro-2-fluorostyryl cyclopropyl ketone (3n) $^{19}$F NMR (376 MHz, CDCl$_3$)
4-(trifluoromethyl)styryl cyclopropyl ketone (3o) \(^1\)H NMR (400 MHz, CDCl\(_3\))

![NMR spectrum](image1)

<table>
<thead>
<tr>
<th>(f_1) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.99</td>
</tr>
<tr>
<td>1.98</td>
</tr>
<tr>
<td>1.01</td>
</tr>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>1.11</td>
</tr>
<tr>
<td>3.97</td>
</tr>
<tr>
<td>0.98</td>
</tr>
<tr>
<td>0.99</td>
</tr>
<tr>
<td>0.998</td>
</tr>
<tr>
<td>1.007</td>
</tr>
<tr>
<td>1.010</td>
</tr>
<tr>
<td>1.018</td>
</tr>
<tr>
<td>1.026</td>
</tr>
<tr>
<td>1.158</td>
</tr>
<tr>
<td>1.166</td>
</tr>
<tr>
<td>1.169</td>
</tr>
<tr>
<td>1.175</td>
</tr>
<tr>
<td>1.178</td>
</tr>
<tr>
<td>1.186</td>
</tr>
<tr>
<td>1.187</td>
</tr>
<tr>
<td>1.196</td>
</tr>
</tbody>
</table>

4-(trifluoromethyl)styryl cyclopropyl ketone (3o) \(^{13}\)C NMR (101 MHz, CDCl\(_3\))

![NMR spectrum](image2)

<table>
<thead>
<tr>
<th>(f_1) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>198.74</td>
</tr>
<tr>
<td>161.11</td>
</tr>
<tr>
<td>19.983</td>
</tr>
<tr>
<td>119.760</td>
</tr>
<tr>
<td>122.467</td>
</tr>
<tr>
<td>125.176</td>
</tr>
<tr>
<td>125.787</td>
</tr>
<tr>
<td>125.825</td>
</tr>
<tr>
<td>125.863</td>
</tr>
<tr>
<td>125.901</td>
</tr>
<tr>
<td>127.883</td>
</tr>
<tr>
<td>128.342</td>
</tr>
<tr>
<td>128.413</td>
</tr>
<tr>
<td>131.196</td>
</tr>
<tr>
<td>131.522</td>
</tr>
<tr>
<td>131.846</td>
</tr>
<tr>
<td>132.171</td>
</tr>
<tr>
<td>138.115</td>
</tr>
<tr>
<td>138.127</td>
</tr>
<tr>
<td>138.142</td>
</tr>
<tr>
<td>138.156</td>
</tr>
<tr>
<td>139.909</td>
</tr>
<tr>
<td>199.740</td>
</tr>
</tbody>
</table>

O

F\(_3\)C

F\(_3\)C
4-(trifluoromethyl)styril cyclopropyl ketone (3o) $^{19}$F NMR (376 MHz, CDCl$_3$)

\[
\begin{align*}
\text{F}_3\text{C} &- \text{O} \\
\end{align*}
\]

4-nitrostyril cyclopropyl ketone (3p) $^1$H NMR (400 MHz, CDCl$_3$)

\[
\begin{align*}
\text{O}_2\text{N} &- \text{O} \\
\end{align*}
\]
4-nitrostyryl cyclopropyl ketone (3p) $^{13}$C NMR (101 MHz, CDCl$_3$)

![NMR Spectrum of 4-nitrostyryl cyclopropyl ketone (3p)]

4-cyanostyryl cyclopropyl ketone (3q) $^1$H NMR (400 MHz, CDCl$_3$)

![NMR Spectrum of 4-cyanostyryl cyclopropyl ketone (3q)]
4-cyanostyryl cyclopropyl ketone (3q) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-methylstyril cyclopropyl ketone (3r) $^1$H NMR (400 MHz, CDCl$_3$)
4-methylstyril cyclopropyl ketone (3r) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-methoxystyril cyclopropyl ketone (3s) $^1$H NMR (400 MHz, CDCl$_3$)
4-methoxystyryl cyclopropyl ketone (3s) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-ethoxystyryl cyclopropyl ketone (3t) $^1$H NMR (400 MHz, CDCl$_3$)
4-ethoxystyryl cyclopropyl ketone (3t) \(^{13}\)C NMR (101 MHz, CDCl\(_3\))

3,4-dimethoxystyryl cyclopropyl ketone (3u) \(^1\)H NMR (400 MHz, CDCl\(_3\))
3,4-dimethoxystyryl cyclopropyl ketone (3u) $^{13}$C NMR (101 MHz, CDCl$_3$)

![3,4-dimethoxystyryl cyclopropyl ketone (3u) $^{13}$C NMR (101 MHz, CDCl$_3$) graph]

3-ethoxy-4-methoxystyryl cyclopropyl ketone (3v) $^1$H NMR (400 MHz, CDCl$_3$)

![3-ethoxy-4-methoxystyryl cyclopropyl ketone (3v) $^1$H NMR (400 MHz, CDCl$_3$) graph]
3-ethoxy-4-methoxystyryl cyclopropyl ketone (3v) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{1}$H NMR (400 MHz, CDCl$_3$)

3,4-methylenedioxystyryl cyclopropyl ketone (3w) $^{1}$H NMR (400 MHz, CDCl$_3$)
3,4-methylenedioxy styryl cyclopropyl ketone (3w) $^{13}$C NMR (101 MHz, CDCl$_3$)

1-(2,3-dihydrobenzofuran-5-yl)vinyl cyclopropyl ketone (3x) $^1$H NMR (400 MHz, CDCl$_3$)
1-(2,3-dihydrobenzofuran-5-yl)vin2-yl cyclopropyl ketone (3x) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-(methylthio)styryl cyclopropyl ketone (3y) $^1$H NMR (400 MHz, CDCl$_3$)
4-(methylthio)styryl cyclopropyl ketone (3y) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-(methylsulfonyl)styryl cyclopropyl ketone (3z) $^1$H NMR (400 MHz, CDCl$_3$)
4-(methylsulfonyl)styryl cyclopropyl ketone (3z) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-(dimethylamino)styryl cyclopropyl ketone (3aa) $^1$H NMR (400 MHz, CDCl$_3$)
4-(dimethylamino)styryl cyclopropyl ketone (3aa) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-morpholinostyryl cyclopropyl ketone (3bb) $^1$H NMR (400 MHz, CDCl$_3$)
4-morpholinostyryl cyclopropyl ketone (3bb) $^{13}$C NMR (101 MHz, CDCl$_3$)

1-(pyridin-4-yl)vin-2-yl cyclopropyl ketone (3cc) $^1$H NMR (400 MHz, CDCl$_3$)
1-(pyridin-4-yl)vin-2-yl cyclopropyl ketone (3cc) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{1}$H NMR (400 MHz, CDCl$_3$)

1-(thiophen-2-yl)vin-2-yl cyclopropyl ketone (3dd) $^{1}$H NMR (400 MHz, CDCl$_3$)
1-(thiophen-2-yl)vin-2-yl cyclopropyl ketone (3dd) $^{13}$C NMR (101 MHz, CDCl$_3$)

2-(1-methylpyrazol-4-yl)vinyl cyclopropyl ketone (3ee) $^1$H NMR (400 MHz, CDCl$_3$)
2-(1-methylpyrazol-4-yl)vinyl cyclopropyl ketone (3ee) $^{13}$C NMR (101 MHz, CDCl$_3$).

[Diagram of 2-(1-methylpyrazol-4-yl)vinyl cyclopropyl ketone (3ee)]

Styryl 1-(ethylcarboxy)cycloprop-1-yl ketone (3ff) $^1$H NMR (400 MHz, CDCl$_3$)

[Diagram of styryl 1-(ethylcarboxy)cycloprop-1-yl ketone (3ff)]
styryl 1-(ethylcarboxy)cycloprop-1-yl ketone (3ff) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-methoxystyryl cyclopropyl ketone oxime (4) $^1$H NMR (400 MHz, CDCl$_3$)
4-methoxystyryl cyclopropyl ketone oxime (4) $^{13}$C NMR (101 MHz, CDCl$_3$)

ethyl 4-chloro-α-cyanocinnamate (5a) $^1$H NMR (400 MHz, CDCl$_3$)
ethyl 4-chloro-α-cyanocinnamate (5a) $^{13}$C NMR (101 MHz, CDCl$_3$)

\[
\begin{array}{c}
\text{O} \\
\text{Cl} \\
\text{O} \\
\text{CN}
\end{array}
\]

ethyl 4-methoxy-α-cyanocinnamate (5b) $^1$H NMR (400 MHz, CDCl$_3$)

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{CN}
\end{array}
\]
ethyl 4-methoxy-α-cyanocinnamate (5b) \(^{13}\)C NMR (101 MHz, CDCl\(_3\))

\[
\begin{align*}
\text{C} & : 14.265 \\
\text{N} & : 55.699 \\
\text{O} & : 62.506 \\
\text{O} & : 76.841 \\
\text{Cl} & : 77.159 \\
\text{Cl} & : 77.478 \\
\text{C} & : 99.323 \\
\text{C} & : 114.824 \\
\text{C} & : 116.313 \\
\text{C} & : 124.389 \\
\text{C} & : 133.717 \\
\text{C} & : 154.485 \\
\text{C} & : 163.192 \\
\text{C} & : 163.842 \\
\end{align*}
\]

4-chlorostyryl methyl sulfide (6a) \(^1\)H NMR (400 MHz, CDCl\(_3\))

\[
\begin{align*}
\text{H} & : 2.04 \\
\text{H} & : 1.96 \\
\text{H} & : 1.549 \\
\text{H} & : 1.07 \\
\text{H} & : 1.02 \\
\text{H} & : 0.92 \\
\text{H} & : 0.81 \\
\text{H} & : 0.75 \\
\text{H} & : 0.65 \\
\text{H} & : 0.45 \\
\text{H} & : 0.35 \\
\text{H} & : 0.25 \\
\text{H} & : 0.15 \\
\text{H} & : 0.05 \\
\end{align*}
\]
4-chlorostyryl methyl sulfide (6a) $^{13}$C NMR (101 MHz, CDCl$_3$)

4-(trifluoromethyl)styryl methyl sulfide (6b) $^1$H NMR (400 MHz, CDCl$_3$)
4-(trifluoromethyl)styryl methyl sulfide (6b) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{13}$C NMR spectrum showing peaks at various ppm values.

4-(trifluoromethyl)styryl methyl sulfide (6b) $^{19}$F NMR (377 MHz, CDCl$_3$)

$^{19}$F NMR spectrum showing peaks at various ppm values.
4-methoxystyryl methyl sulfide (6c) $^1$H NMR (400 MHz, CDCl$_3$)

4-methoxystyryl methyl sulfide (6c) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-methoxystyryl methyl sulfoxide (7) $^1$H NMR (400 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$)

4-methoxystyryl methyl sulfoxide (7) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
ethyl 3-(4-chlorophenyl)-3-oxopropanedithioate (8a)

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{S} & \quad \text{S}
\end{align*}
\]

1H (ppm)


13C (ppm)

12.870 27.980 76.730 CDCl₃ 77.048 CDCl₃ 77.365 CDCl₃ 107.684 127.938 129.079 132.796 137.982 168.121 216.900

ethyl 3-(4-chlorophenyl)-3-oxopropanedithioate (8a)

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{S} & \quad \text{S}
\end{align*}
\]

1H (ppm)


13C (ppm)

12.870 27.980 76.730 CDCl₃ 77.048 CDCl₃ 77.365 CDCl₃ 107.684 127.938 129.079 132.796 137.982 168.121 216.900
$1^-(4$-chlorophenyl)-3,3-bis(ethylthio)prop-2-en-1-one (8b) $^1$H NMR (400 MHz, CDCl$_3$)

$1^-(4$-chlorophenyl)-3,3-bis(ethylthio)prop-2-en-1-one (8b) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-(4-chlorophenyl)-5-(ethylthio)isothiazole (9) £H NMR (400 MHz, CDCl$_3$)

3-(4-chlorophenyl)-5-(ethylthio)isothiazole (9) £C NMR (101 MHz, CDCl$_3$)
3-(4-chlorophenyl)-5-(ethylthio)isoxazole (10) $^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum](image1)

3-(4-chlorophenyl)-5-(ethylthio)isoxazole (10) $^{13}$C NMR (101 MHz, CDCl$_3$)

![NMR spectrum](image2)
5(3)-(4-chlorophenyl)-3(5)-(ethylthio)pyrazole (11) $^1$H NMR (400 MHz, CDCl$_3$)

5(3)-(4-chlorophenyl)-3(5)-(ethylthio)pyrazole (11) $^{13}$C NMR (101 MHz, CDCl$_3$)
4-chloro-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (12a) $^1$H NMR (400 MHz, CDCl$_3$)

4-chloro-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (12b) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-(4-chlorophenyl)-5-(ethylthio)-4-iodopyrazole (12b) $^1$H NMR (400 MHz, CDCl$_3$)

3-(4-chlorophenyl)-5-(ethylthio)-4-iodopyrazole (1b) $^{13}$C NMR (101 MHz, CDCl$_3$)
$3$-(4-chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a) $^1$H NMR (600 MHz, CDCl$_3$)

$3$-(4-chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a) $^{13}$C NMR (151 MHz, CDCl$_3$)
3-(4-chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a) HMBC (151 MHz, CDCl₃)

5-(4-chlorophenyl)-3-(ethylthio)-1-methylpyrazole (13a') ¹H NMR (600 MHz, CDCl₃)
3-(4-chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a') $^{13}$C NMR (151 MHz, CDCl$_3$)

3-(4-chlorophenyl)-5-(ethylthio)-1-methylpyrazole (13a') HMBC (600 MHz, CDCl$_3$)
1-(carbamethoxy)methyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13b) $^1$H NMR (600 MHz, CDCl$_3$)

1-(carbamethoxy)methyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13b) $^{13}$C NMR (151 MHz, CDCl$_3$)
1-acetyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13c) $^1$H NMR (400 MHz, CDCl$_3$)

1-acetyl-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13c) $^{13}$C NMR (101 MHz, CDCl$_3$)
1-carbethoxy-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13d) \(^1\)H NMR (400 MHz, CDCl\(_3\))

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{O} \\
\text{S} \\
\text{O}
\end{array}
\]

1-carbethoxy-3-(4-chlorophenyl)-5-(ethylthio)pyrazole (13d) \(^13\)C NMR (101 MHz, CDCl\(_3\))

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{O} \\
\text{S} \\
\text{O}
\end{array}
\]
5(3)-(4-chlorophenyl)-3(5)-(ethylsulfinyl)pyrazole (14) $^1$H NMR (400 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$)

5(3)-(4-chlorophenyl)-3(5)-(ethylsulfinyl)pyrazole (14) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
3-phenylisoxazol-5-one (15) $^1$H NMR (400 MHz, CDCl$_3$)

3-phenylisoxazol-5-one (15) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-phenyl-5-chloroisoxazole (16) $^1$H NMR (400 MHz, CDCl$_3$)

3-phenyl-5-chloroisoxazole (16) $^{13}$C NMR (101 MHz, CDCl$_3$)
CHAPTER 7.  ONE-POT SYNTHESIS OF KETENE DITHIOACETALS FROM ALDEHYDES AND DISULFIDES VIA AN IN SITU HORNER-WADSWORTH-EMMONS REACTION

James S. Klimavicz\textsuperscript{1,2,*} and Joel R. Coats\textsuperscript{1}

\textsuperscript{1}Department of Entomology, Iowa State University, Ames, IA, 50011
\textsuperscript{2}Department of Chemistry, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to \textit{Tetrahedron Letters}.

Abstract

Ketene dithioacetals (KDTAs) are important intermediates in chemical synthesis, serving as intermediates in carbonyl homologation and heterocycle synthesis, and undergoing nucleophilic or electrophilic attack. While \(\alpha\)-carbonylketene dithioacetals and other ketene dithioacetals containing electron-withdrawing groups are readily prepared, KDTAs lacking such a functionality are more difficult to synthesize. Herein, we describe a one-pot synthesis of KDTAs from aldehydes, dimethyl methylphosphonate, and dialkyl disulfides. A bis(alkylthio)methylphosphonate is formed \textit{in situ}, which then undergoes a Horner-Wadsworth-Emmons reaction with the aldehyde to produce KDTAs in moderate to good yields, typically ranging from 54-86%.

Keywords: Ketene dithioacetal, Vinyl sulfide, Thioacetal, Horner-Wadsworth-Emmons

Introduction

Ketene dithioacetals are valuable building blocks in organic synthesis,\textsuperscript{1-4} serving as intermediates for the synthesis of various derivatized benzenes,\textsuperscript{5-6} azoles,\textsuperscript{7-11} azines,\textsuperscript{12-15} pyrones,\textsuperscript{16} and other heterocycles;\textsuperscript{17-18} as masked carbonyl moieties or carbonyl homologation

\textsuperscript{*} JSK optimized the reaction and designed, synthesized, purified, and characterized all compounds and wrote the manuscript. JRC assisted in the revision of the manuscript.
intermediates,\textsuperscript{19-24} or as nucleophilic building blocks.\textsuperscript{25-26} Ketene dithioacetals possessing an electron-withdrawing group (EWG)—including esters, nitriles, ketones, phosphonates, sulfoxides, or nitro groups—on the \( \alpha \)-carbon are frequently encountered due to their ease of synthesis from the base-mediated addition of carbon disulfide to an activated carbon, followed by the addition of an alkyl halide.\textsuperscript{27} Despite their utility, ketene dithioacetals without an \( \alpha \)-EWG (KDTAs) are less prevalent. KDTAs have been used as reagents in a variety of cycloadditions, including inverse electron demand Diels-Alder reactions with substrates including pyrones,\textsuperscript{28} tropone,\textsuperscript{29} and \( \alpha,\beta \)-unsaturated carbonyls,\textsuperscript{30} the aza-Diels-Alder reaction,\textsuperscript{31} titanium-catalyzed [2+2] cycloadditions,\textsuperscript{32} and [3+2] dipolar additions with nitrile oxides and nitrones.\textsuperscript{33} Additionally, KDTAs have served as precursors to electron-deficient ketene dithioacetal \( S,S' \)-dioxides,\textsuperscript{34} difluoroxyethylenemethylene-bridge compounds via oxidative alkoxydifluorodesulfuration,\textsuperscript{35} and amines and cyclic amino acids from the addition to azides.\textsuperscript{36}

Methods for the synthesis of KDTAs are somewhat limited. The most common method is the Horner-Wadsworth-Emmons (HWE) reaction using dialkyl bis(alkylthio)methylphosphonates (DBMPs) (Figure 7.1a,b);\textsuperscript{37-38} Alternatively, these compounds may be prepared from 2-trimethylsilyl-1,3-dithiane via Peterson olefination (Figure 7.1c),\textsuperscript{39} from chloroalkyl alkanedithioates (Figure 7.1d),\textsuperscript{40} from \( \alpha \)-chlorodithioacetals,\textsuperscript{41} or from the corresponding 1,1-dibromoalkenes and the appropriate thiol using excess DBU in DMSO (Figure 7.1e).\textsuperscript{42} While the HWE method for forming KDTAs is attractive due to its simplicity and generally high yields, DBMPs are not readily available. Even the simplest DBMP, dimethyl bis(methylthio)methylphosphonate, is not readily commercially available, and must be synthesized by the Arbuzov reaction of triethyl phosphate with bis(methylthio)chloromethane, the latter of which also lacks commercial availability.\textsuperscript{43} DBMPs have also been synthesized
previously from methylthiomethylphosphonates, and there is a single instance of a multi-step, one-pot reaction of the synthesis of diethyl bis(phenylthio)methylphosphonate from diethyl methylphosphonate, produced by alternating the addition of butyllithium and diphenyl disulfide. The latter approach to the synthesis of DBMPs is attractive because methylphosphonates are readily available, and avoid the necessity of synthesizing chlorodithioacetals. By simplifying the one-pot synthesis of DBMPs over the previous procedure, the work herein allows for the synthesis of KDTAs from dimethyl methylphosphonate (1), disulfides, and aldehydes in one pot.
Results and Discussion

Because sulfur stabilizes carbanions, we began our approach to the synthesis of KDTAs by attempting to form the anion of dimethyl bis(ethylthio)methylphosphonate (6) using 1, diethyl disulfide (2), and an excess of lithium diisopropylamide (Scheme 7.1), under the premise that sulfenylation occurs stepwise, and that the rate of addition of diethyl disulfide is fastest for the phosphonate anion 1⁻, followed by the monosulfenylated phosphonate 5, and the bis(sulfenyl)ated phosphonate anion 6 adds most slowly to diethyl disulfide. This pattern follows

Table 7.1. Optimization of the reaction conditions. Standard conditions: 1 (1.1 mmol), LDA (3.3 mmol), diethyl disulfide (2, 2.2 mmol) added dropwise, in THF (15 mL) at 0 ºC, then benzaldehyde (3, 1 mmol), warming to 55 ºC.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Change from standard conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No change</td>
<td>82%</td>
</tr>
<tr>
<td>2</td>
<td>Et₂O instead of THF</td>
<td>43%</td>
</tr>
<tr>
<td>3</td>
<td>LiTMP instead of LDA</td>
<td>76%</td>
</tr>
<tr>
<td>4</td>
<td>nBuLi instead of LDA</td>
<td>trace</td>
</tr>
<tr>
<td>5</td>
<td>Rapid addition of EtSSEt</td>
<td>18%</td>
</tr>
<tr>
<td>6</td>
<td>3 equivalents of 2</td>
<td>46%</td>
</tr>
<tr>
<td>7</td>
<td>Step 1 at 0 ºC</td>
<td>31%</td>
</tr>
<tr>
<td>8</td>
<td>Step 2 at 78 ºC</td>
<td>80%</td>
</tr>
<tr>
<td>9</td>
<td>Warming to 22 ºC instead of 55 ºC in step 2</td>
<td>67%</td>
</tr>
</tbody>
</table>

Scheme 7.1. Stepwise synthesis of 4a from dimethyl methylphosphonate (1).
that expected from basicity and sterics. The addition of benzaldehyde (3) to a solution of the anion 6 then forms the phosphonoalkoxide 7, the expected HWE intermediate, which readily eliminates dimethylphosphate to give the expected KDTA.

Optimization of the reaction conditions is shown in Table 7.1. Changing the solvent from THF to diethyl ether (entry 2) significantly decreased the yield, likely a result of the observed decrease of the solubility of anion 1· in diethyl ether. Replacing LDA with lithium 2,2,6,6-tetramethylpiperide (entry 3) resulted in a slight reduction of yield, while using butyllithium (entry 4) as the base produced only a trace of 4a, and a large quantity of ethyl butyl sulfide was detected by GCMS, as expected from the direct addition of butyllithium to diethyl disulfide. The yield was dramatically reduced if the diethyl disulfide was added rapidly to the reaction (entry 5), or if an excess of 2 was used (entry 6). Conceivably, using an excess of 2 may result in the formation of trithioorthoester 8 via the additional sulfonylation of 6 (Scheme 7.1); however, no effort was made to synthesize or isolate 8 from the reaction. Additionally, using excess 2a or attempting the formation of the anion 6 was attempted at 0 ºC (entry 7) resulted in the production
of substantial quantities of \(N\)-(ethylthio)diisopropylamine, as detected in the reaction mixture by GCMS. No benefit was seen when the reaction was cooled to -78 ºC before the addition of the aldehyde (entry 8). While the elimination of dimethylphosphate to form 4a occurs readily, warming briefly to 55 ºC is beneficial for completing the elimination, as omitting this warming step lowered the reaction yield (entry 9).

Scheme 7.2 shows the scope of the one-pot reaction for synthesizing a variety of ketene dithioacetals. In general, KDTAs containing electron-withdrawing groups (e.g. 4b-g,i) were produced in superior yields to those with electron-donating groups (e.g. 4k and 4l). The lower yield of the nitro derivative 4h may be a result of a competing side reactions occurring at the nitro group.\(^{47}\) Both \(\pi\)-excessive and \(\pi\)-deficient heterocyclic carboxaldehydes produced their respective products (4t-v) in acceptable yields, as did both \(\alpha,\beta\)-unsaturated aldehyde cinnamaldehyde (4w) and alkyl aldehyde citronellal (4x).

The use of several disulfides was also explored. While the optimization of conditions was performed using diethyl disulfide, dimethyl disulfide also gave acceptable yields of the expected products (4m and 4s). When using diisopropyl disulfide to produce 4d, the expected product was obtained; however, the yield was substantially lower than that achieved when synthesizing the ethyl analog 4c, likely due to steric hinderance of the phosphonate anion. Diphenyl disulfide (2d) also gave acceptable yields of 4e. It is unclear why the yield of 4n was so poor, especially when compared to 4m or 4o; despite several attempts using freshly-distilled 4-trifluoromethoxybenzaldehyde, we could not achieve an acceptable yield of this product.

We also explored the use of \(S\)-methyl methanethiosulfonate (MeSSO\(_2\)Me) and dimethyl disulfide as sulfenylation reagents, as thiosulfonates are generally considered more reactive and effective than disulfides for sulfenylation.\(^{48-49}\) However, under the normal reaction conditions, a
heavy white precipitate, likely lithium methanesulfinate, formed, which interfered with stirring and resulted in almost no product; a trace of $4s$ was detected by GCMS when attempting to synthesize this compound using MeSSO$_2$Me in place of dimethyl disulfide, compared to the respectable yield of 59%.

The purified KDTAs typically have little odor and appear to be stable for at least several months when stored under argon in the dark at room temperature; however, we opted to store the isolated compounds at -20 ºC. If these compounds are left on the benchtop exposed to air and light for several days, they slowly yellow and become malodorous, and the NMRs of these samples show minor degradation.

**Conclusions**

Although ketenedithioacetals have been readily synthesized from EWG-stabilized carbanions in the presence of alkyl halides and carbon disulfide, the method described here permits the one-pot synthesis of KDTAs from aldehydes via a Horner-Wadsworth-Emmons reaction using readily-available dimethyl methylphosphonate and disulfides. Benzaldehydes containing electron-withdrawing substituents tended to result in somewhat better yields; however, the yield of the KDTA products were also acceptable for electron-rich aldehydes. Additionally, heterocyclic, allylic, and alkyl aldehydes were all smoothly converted to KDTAs. This simple strategy for the synthesis of ketenedithioacetals should improve the accessibility of this interesting class of molecules for use in organic synthesis.

**Acknowledgements**

The authors would like to thank the Dr. Peter Porpiglia and AMVAC Chemical Corporation for funding this research, and Dr. George Kraus for his review of this paper. We also wish to thank Drs. Sarah Cady, Shu Xu, and Kamel Harrata for training and assistance in the Iowa State University Chemical Instrumentation Facility.
Experimental

General information

Anhydrous tetrahydrofuran was purchased from Acros Organics and was stored under argon. Diisopropylamine was purchased from Sigma-Aldrich and distilled under argon before use. \(n\)-Butyllithium was purchased from Sigma-Aldrich and titrated before use. Dimethyl methylphosphonate was purchased from Strem Chemicals and was distilled under vacuum before use. All disulfides were purchased from TCI and were used as received, and aldehydes were purchased from multiple sources, including Sigma-Aldrich, Acros Organics, Alfa Aesar, Matrix Scientific, Oakwood Chemicals, Combi-Blocks, Synthonix, and Chem-Impex. Liquid aldehydes were purified by distillation or column chromatography before use to remove any carboxylic acid. All reactions were performed under an argon atmosphere. Ketenedithioacetals were purified on a Buchi Pure C-810 Flash chromatography system using HPLC grade solvents on 12 g or 25 g FlashPure silica cartridges. The characterization of all compounds was performed at the Iowa State University Chemical Instrumentation Facility. NMR spectra were obtained using Avance III 600 MHz and Avance NEO 400 MHz spectrometers. Chemical shifts are reported in ppm relative to the residual solvent peak (CDCl\(_3\): 7.26 ppm for \(^1\)H and 77.16 ppm for \(^{13}\)C; DMSO-\(d_6\): 2.50 for \(^1\)H and 39.52 ppm for \(^{13}\)C; CD\(_3\)OD: 3.31 ppm for \(^1\)H and 49.00 ppm for \(^{13}\)C) or, for \(^{19}\)F, an external CFCl\(_3\) reference (0 ppm). Coupling constants are reported in Hz.

HRMS analysis was performed using electrospray ionization positive ion mode (ESI\(^+\)) on an Agilent QTOF 6540 mass spectrometer. Accurate mass measurement was achieved by constantly infusing a calibrant (masses: 121.0508 and 922.0098). Melting points were obtained on Stuart SMP30 melting point apparatus using a temperature ramp rate of 3 °C min\(^{-1}\).

All reactions should be performed in a fumehood, as dialkyl disulfides are foul-smelling compounds. To minimize the risk of the production of free thiol, the aqueous workup should be
performed in the order described in the general procedure, with basic washings taking place before acidic washings; the basic and acidic washings should not be mixed. Thiolates in the basic washing may be destroyed by the careful addition of sodium hypochlorite.

**General Procedure for the Synthesis of Ketenedithioacetals**

A 50 mL 14/20 one-neck round bottom flask with rubber septum and stir bar was flushed with argon, and charged with diisopropylamine (344 mg, 3.4 mmol) and tetrahydrofuran (15 mL). The solution was cooled to -20 ºC, and n-butyllithium (nominally 2.5 M in hexane, 3.3 mmol) was added over three minutes. The LDA solution was stirred at -20 ºC for 10 minutes, and dimethyl methylphosphonate (136 mg, 1.1 mmol) was added over 1 minute, and the solution was stirred -20 ºC for 10 minutes. The solution was cooled to -78 ºC, diethyl disulfide (147 mg, 1.2 mmol) was added, and the solution was stirred at -78 ºC for 15 minutes, and then warmed to 0 ºC, followed by stirring for 30 minutes. The aldehyde was dissolved in tetrahydrofuran (5 mL) and added over 3 minutes at 0 ºC, followed by stirring at 0 ºC. The reaction progress was monitored by TLC, using 2,4-dinitrophenylhydrazine stain to check for the presence of aldehyde. After consumption of the aldehyde, the reaction was warmed to 55 ºC for 15 minutes, cooled to room temperature, and then quenched by the addition of saturated ammonium chloride solution. The organic layer was diluted by the addition of hexane (20 mL), and was washed subsequently with 0.5 M sodium hydroxide (2 x 20 mL), water (2 x 20 mL), 0.5 M hydrochloric acid (20 mL), and brine (20 mL), and then dried over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the crude product was purified by flash chromatography on silica using a gradient of 100:0 to 95:5 hexane:ethyl acetate as an eluent.

**S,S'-diethyl phenylketenedithioacetal (4a)** Colorless oil. Yield: 184 mg (82%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66 – 7.58 (m, 1H), 7.38 – 7.29 (m, 2H), 7.24 (dd, $J = 7.4, 1.9$ Hz, 1H), 7.02 (s, 1H), 2.851 (q, $J = 7.3$ Hz, 2H), 2.850 (q, $J = 7.3$ Hz, 2H), 1.32 (t, $J = 7.3$ Hz, 3H),
1.24 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 136.6, 135.0, 132.2, 129.5, 128.2, 127.4, 28.1, 27.8, 15.0, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{17}$S$_2^+$ 225.0766; found: 225.0766.

$S,S'$-diethyl (4-fluorophenyl)ketenedithioacetal (4b) Colorless oil. Yield: 205 mg (85%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.66 – 7.55 (m, 2H), 7.07 – 6.98 (m, 2H), 6.98 (s, 1H), 2.85 (q, $J = 7.3$ Hz, 3H), 2.84 (q, $J = 7.3$ Hz, 2H), 1.31 (t, $J = 7.3$ Hz, 3H), 1.23 (t, $J = 7.4$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 161.8 (d, $J = 247.8$ Hz), 134.0, 132.7 (d, $J = 3.5$ Hz), 132.0 (d, $J = 2.0$ Hz), 131.2 (d, $J = 7.9$ Hz), 115.1 (d, $J = 21.4$ Hz), 28.1, 27.8, 15.0, 14.4. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -113.9 (tt, $J = 8.9$, 5.7 Hz). HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{16}$FS$_2^+$ 243.0672; found: 243.0668.

$S,S'$-diethyl (4-chlorophenyl)ketenedithioacetal (4c) Colorless oil. Yield: 206 mg (80%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60 – 7.52 (m, 2H), 7.32 – 7.27 (m, 2H), 6.93 (s, 1H), 2.847 (q, $J = 7.3$ Hz, 2H), 2.844 (q, $J = 7.3$ Hz, 2H), 1.31 (t, $J = 7.3$ Hz, 3H), 1.23 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 135.1, 133.4, 133.3, 132.9, 130.7, 128.3, 28.2, 27.8, 15.0, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{16}$ClS$_2^+$ 259.0376; found: 259.0377.

$S,S'$-diisopropyl (4-chlorophenyl)ketenedithioacetal (4d) Eluent: 100:0 to 98:2 hexane:ethyl acetate. Colorless oil. Yield: 122 mg (43%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.63 – 7.55 (m, 2H), 7.33 – 7.25 (m, 2H), 6.99 (s, 1H), 3.53 (hept, $J = 6.7$ Hz, 1H), 3.42 (hept, $J = 6.7$ Hz, 1H), 1.32 (d, $J = 6.7$ Hz, 6H), 1.27 (d, $J = 6.7$ Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 135.5, 135.1, 133.5, 133.0, 130.9, 128.3, 38.2, 37.3, 23.1, 22.7. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{14}$H$_{20}$ClS$_2^+$ 287.0689; found: 287.0692.

$S,S'$-diphenyl (4-chlorophenyl)ketenedithioacetal (4e) Recrystallized from hot acetonitrile to yield white prisms (202 mg, 57%). mp 79 – 81 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ
7.59 – 7.54 (m, 1H), 7.36 – 7.29 (m, 6H), 7.29 – 7.23 (m, 4H), 7.04 (s, 1H). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \(\delta 135.6, 134.4, 133.7, 133.58, 133.55, 132.68, 132.64, 131.1, 130.6, 129.2, 128.9, 128.5, 128.2, 127.4. HRMS (+ESI-QTOF) \(m/z\): [M+H]+ calcd for C\(_{20}\)H\(_{16}\)ClS\(_2^+\) 355.0376; found: 355.0375.

\(S,S^{'}\)-diethyl (4-bromophenyl)ketenedithioacetal (4f) Colorless oil. Yield: 226 mg (75%). \(^1\text{H NMR (400 MHz, CDCl}_3\) \(\delta 7.52 – 7.47 (m, 2H), 7.46 – 7.42 (m, 2H), 6.90 (s, 1H), 2.846 (q, \(J = 7.3\) Hz, 2H), 2.844 (q, \(J = 7.3\) Hz, 2H), 1.31 (t, \(J = 7.3\) Hz, 3H), 1.23 (t, \(J = 7.3\) Hz, 3H). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \(\delta 135.5, 133.6, 133.2, 131.3, 131.0, 121.1, 28.2, 27.8, 15.0, 14.3. HRMS (+ESI-QTOF) \(m/z\): [M+H]+ calcd for C\(_{12}\)H\(_{16}\)BrS\(_2^+\) 302.9871; found: 302.9875.

\(S,S^{'}\)-diethyl (4-(trifluoromethyl)phenyl)ketenedithioacetal (4g) Colorless oil. Yield: 229 mg (78%). \(^1\text{H NMR (400 MHz, CDCl}_3\) \(\delta 7.74 – 7.67 (m, 2H), 7.61 – 7.53 (m, 2H), 6.95 (s, 1H), 2.873 (q, \(J = 7.3\) Hz, 2H), 2.867 (q, \(J = 7.3\) Hz, 2H), 1.33 (t, \(J = 7.3\) Hz, 3H), 1.24 (t, \(J = 7.3\) Hz, 3H). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \(\delta 140.1 (q, J_{CF} = 1.3\) Hz), 135.9, 132.0, 129.5, 128.8 (q, \(J_{CF} = 32.4\) Hz), 125.1 (q, \(J_{CF} = 3.8\) Hz), 124.4 (q, \(J_{CF} = 270.2\) Hz), 28.3, 27.9, 15.0, 14.3. \(^{19}\text{F NMR (377 MHz, CDCl}_3\) \(\delta -62.5. HRMS (+ESI-QTOF) \(m/z\): [M+H]+ calcd for C\(_{13}\)H\(_{16}\)F\(_3\)S\(_2^+\) 293.0640; found: 293.0637.

\(S,S^{'}\)-diethyl (4-nitrophenyl)ketenedithioacetal (4h) Eluent: 100:0 to 85:5 hexane:ethyl acetate. Yellow-red oil that solidifies upon cooling to -20 °C. Yield: 158 mg (59%). \(^1\text{H NMR (400 MHz, CDCl}_3\) \(\delta 8.22 – 8.14 (m, 2H), 7.79 – 7.72 (m, 2H), 6.89 (s, 1H), 2.90 (q, \(J = 7.3\) Hz, 4H), 1.34 (t, \(J = 7.4\) Hz, 3H), 1.26 (t, \(J = 7.3\) Hz, 3H). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \(\delta 146.1, 143.1, 139.2, 129.8, 129.7, 123.6, 28.6, 28.1, 15.1, 14.1. HRMS (+ESI-QTOF) \(m/z\): [M+H]+ calcd for C\(_{12}\)H\(_{16}\)NO\(_2\)S\(_2^+\) 270.0617; found: 270.0616.
**S,S'-diethyl (4-cyanophenyl)ketenedithioacetal (4i)** Eluent: 100:0 to 85:5 hexane:ethyl acetate. Yellow oil that solidifies upon cooling to -20 ºC. Yield: 204 mg (82%). $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.73 – 7.68 (m, 2H), 7.62 – 7.57 (m, 2H), 6.87 (s, 1H), 2.880 (q, $J = 7.3$ Hz, 2H), 2.877 (q, $J = 7.3$ Hz, 2H), 1.32 (t, $J = 7.3$ Hz, 3H), 1.24 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 141.0, 137.9, 132.0, 130.7, 129.8, 119.2, 110.1, 28.5, 28.0, 15.0, 14.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{16}$NS$_2^+$ 250.0719; found: 250.0716.

**S,S'-diethyl (4-tolyl)ketenedithioacetal (4j)** Colorless oil. Yield: 206 mg (86%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.56 – 7.49 (m, 2H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.01 (s, 1H), 2.848 (q, $J = 7.3$ Hz, 2H), 2.839 (q, $J = 7.3$ Hz, 2H), 2.35 (s, 3H), 1.31 (t, $J = 7.3$ Hz, 3H), 1.23 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 137.3, 135.5, 133.8, 131.0, 129.5, 128.9, 28.0, 27.8, 21.4, 15.0, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{19}$S$_2^+$ 239.0923; found: 239.0928.

**S,S'-diethyl (4-methoxyphenyl)ketenedithioacetal (4k)** Eluent: 100:0 to 85:5 hexane:ethyl acetate. Colorless oil. Yield: 181 mg (71%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.65 – 7.57 (m, 2H), 7.01 (s, 1H), 6.91 – 6.83 (m, 2H), 3.82 (s, 3H), 2.846 (q, $J = 7.3$ Hz, 2H), 2.833 (q, $J = 7.3$ Hz, 2H), 1.30 (t, $J = 7.3$ Hz, 3H), 1.23 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 158.9, 135.7, 131.0, 129.4, 129.3, 113.6, 55.4, 28.0, 27.8, 15.0, 14.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{13}$H$_{19}$OS$_2^+$ 255.0872; found: 255.0875.

**S,S'-diethyl (4-(methylthio)phenyl)ketenedithioacetal (4l)** Colorless oil. Yield: 184 mg (68%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.61 – 7.53 (m, 2H), 7.23 – 7.14 (m, 2H), 6.96 (s, 1H), 2.849 (q, $J = 7.3$, 2H), 2.835 (q, $J = 7.3$, 2H), 2.49 (s, 3H), 2.84 (q, $J = 7.3$, 2H), 2.49 (s, 3H), 1.30 (t, $J = 7.3$ Hz, 3H), 1.23 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 137.7, 134.6,
133.4, 131.6, 129.9, 126.0, 28.2, 27.8, 15.8, 15.0, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{13}H_{19}S_{3}^+ 271.0643; found: 271.0642.

**S,S'-dimethyl (4-(difluoromethoxy)phenyl)ketenedithioacetal (4m)** Colorless oil.
Yield: 141 mg (54%). ¹H NMR (600 MHz, CDCl₃) δ 7.59 – 7.54 (m, 2H), 7.11 – 7.03 (m, 2H), 6.73 (s, 1H), 6.51 (t, J = 7.40 Hz, 1H), 2.41 (s, 3H), 2.36 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 149.9, 135.9, 133.8, 130.5, 128.5, 119.0, 115.9 (t, J = 259.5 Hz), 17.4, 17.2. ¹⁹F{¹H} NMR (565 MHz, CDCl₃) δ -80.7. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{11}H_{13}F₂O₂S²⁺: 263.0370; found: 263.0372.

**S,S'-dimethyl (4-(trifluoromethoxy)phenyl)ketenedithioacetal (4n)** Colorless oil.
Yield: 34.2 mg (12%). ¹H NMR (600 MHz, CDCl₃) δ 7.62 – 7.55 (m, 1H), 7.22 – 7.13 (m, 1H), 6.71 (s, 1H), 2.41 (s, 3H), 2.36 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 143.5, 136.9, 135.2, 130.5, 128.0, 120.7, 17.5, 17.3. Not observed: -OCF₃. ¹⁹F{¹H} NMR (565 MHz, CDCl₃) δ -57.8.

**S,S'-diethyl (4-(trifluoromethylthio)phenyl)ketenedithioacetal (4o)** Colorless oil.
Yield: 197 mg (61%). ¹H NMR (400 MHz, CDCl₃) δ 7.71 – 7.62 (m, 2H), 7.63 – 7.56 (m, 2H), 6.92 (s, 1H), 2.875 (q, J = 7.3 Hz, 2H), 2.869 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H), 1.25 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 139.2, 136.1, 135.8, 132.1, 130.3, 129.7 (q, J_{CF} = 308.1 Hz), 122.5 (q, J_{CF} = 2.2 Hz), 28.3, 28.0, 15.1, 14.3. ¹⁹F NMR (376 MHz, CDCl₃) δ -42.7. HRMS (+ESI-QTOF) m/z: [M+H]^+ calcd for C_{13}H_{16}F₃S₃^+ 325.0361; found: 325.0363.

**S,S'-diethyl (4-(dimethylamino)phenyl)ketenedithioacetal (4p)** Eluent: 100:0 to 85:5 hexane:ethyl acetate. Pale yellow oil. Yield: 173 mg (65%). ¹H NMR (400 MHz, CDCl₃) δ 7.67 – 7.58 (m, 2H), 7.02 (s, 1H), 6.72 – 6.64 (m, 2H), 2.98 (s, 6H), 2.85 (q, J = 7.3 Hz, 2H), 2.80 (q, J = 7.3 Hz, 2H), 1.28 (t, J = 7.3 Hz, 3H), 1.24 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ
149.8, 137.5, 130.9, 125.9, 125.0, 111.7, 40.5, 28.0, 27.8, 15.0, 14.5. HRMS (+ESI-QTOF) m/z: 
[M+H]$^+$ calcd for C$_{14}$H$_{22}$NS$_2^+$ 268.1188; found: 268.1186.

$\text{S, S'}$-diethyl (3,4-methylenedioxyphenyl)ketenedithioacetal (4q) Eluent: 100:0 to 85:5 hexane:ethyl acetate. Pale yellow oil. Yield: 185 mg (69%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.40 (d, $J = 1.7$ Hz, 1H), 6.99 (dd, $J = 8.1$, 1.5 Hz, 1H), 6.95 (s, 1H), 6.78 (d, $J = 8.1$ Hz, 1H), 5.96 (s, 2H), 2.85 (q, $J = 7.3$ Hz, 2H), 2.81 (q, $J = 7.3$ Hz, 2H), 1.29 (t, $J = 7.3$ Hz, 3H), 1.24 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 147.4, 146.9, 135.4, 130.9, 130.1, 124.2, 109.4, 108.1, 101.2, 28.1, 27.8, 15.0, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{13}$H$_{17}$O$_2$S$_2^+$ 269.0665; found: 269.0665.

$\text{S, S'}$-diethyl (3,4-(difluoromethylenedioxy)phenyl)ketenedithioacetal (4r) Pale yellow oil. Yield: 216 mg (71%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60 (d, $J = 1.7$ Hz, 1H), 7.19 (dd, $J = 8.4$, 1.8 Hz, 1H), 7.00 (d, $J = 8.3$ Hz, 1H), 6.94 (s, 1H), 2.863 (q, $J = 7.3$ Hz, 2H), 2.838 (q, $J = 7.3$ Hz, 2H), 1.30 (t, $J = 7.3$ Hz, 3H), 1.24 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 143.7, 142.7, 133.3, 133.2, 132.9, 131.8 (t, $J_{CF} = 250.0$ Hz), 125.6, 110.2, 109.0, 28.2, 27.8, 15.1, 14.4. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -50.1. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{13}$H$_{15}$F$_2$O$_2$S$_2^+$ 305.0476; found: 305.0477.

$\text{S, S'}$-dimethyl (naphth-1-yl)ketenedithioacetal (4s) Eluent: 100:0 to 90:10 hexane:ethyl acetate. Colorless oil. Yield: 158 mg (59%). Yield: 184 mg (75%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.01 – 7.94 (m, 1H), 7.90 – 7.83 (m, 1H), 7.83 – 7.76 (m, 1H), 7.57 (dt, $J = 7.1$, 1.1 Hz, 1H), 7.56 – 7.44 (m, 3H), 7.22 (s, 1H), 2.53 (s, 3H), 2.23 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 137.7, 133.7, 133.5, 131.6, 128.5, 127.8, 127.3, 126.9, 126.0, 125.8, 125.2, 124.5, 17.4, 17.2. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{14}$H$_{15}$S$_2^+$ 247.0610; found: 247.0606.
\( S,S' \)-diethyl thiophen-2-ylketenedithioacetal (4t) Colorless oil. Yield: 160 mg (60%).

\( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.28 – 7.24 (m, 1H), 7.13 (dd, \( J = 3.8, 1.2 \) Hz, 1H), 6.99 (dd, \( J = 5.3, 3.6 \) Hz, 1H), 2.91 (q, \( J = 7.3 \) Hz, 3H), 2.82 (q, \( J = 7.3 \) Hz, 3H), 1.29 (t, \( J = 7.3 \) Hz, 6H). \( ^{13}C \) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 140.2, 130.7, 129.8, 128.7, 126.6, 126.1, 28.6, 27.7, 15.0, 14.6. HRMS (+ESI-QTOF) \( m/z \): [M+H]\(^+\) calcd for C\(_{10}\)H\(_{15}\)S\(_3\)\(^+\) 231.0330; found: 231.0326.

\( S,S' \)-diethyl (1-methylindol-3-yl)ketenedithioacetal (4u) Eluent: 100:0 to 85:5 hexane:ethyl acetate. Colorless oil. Yield: 187 mg (67%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.99 (s, 1H), 7.72 (dt, \( J = 7.9, 1.0 \) Hz, 1H), 7.40 (d, \( J = 0.7 \) Hz, 1H), 7.31 (dt, \( J = 8.2, 1.1 \) Hz, 1H), 7.26 (ddd, \( J = 8.2, 6.8, 1.1 \) Hz, 1H), 7.18 (ddd, \( J = 8.0, 6.9, 1.2 \) Hz, 1H), 3.83 (s, 3H), 2.94 (q, \( J = 7.3 \) Hz, 2H), 2.82 (q, \( J = 7.3 \) Hz, 2H), 1.31 (t, \( J = 7.3 \) Hz, 3H), 1.27 (t, \( J = 7.3 \) Hz, 3H). \(^{13}C \) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 136.2, 130.2, 129.3, 128.1, 124.1, 122.3, 120.0, 118.6, 111.9, 109.4, 33.3, 28.3, 27.8, 15.3, 14.6. HRMS (+ESI-QTOF) \( m/z \): [M+H]\(^+\) calcd for C\(_{15}\)H\(_{20}\)NS\(_2\)\(^+\) 278.1032; found: 278.1031.

\( S,S' \)-diethyl (2-chloropyridin-5-yl)ketenedithioacetal (4v) Eluent: 100:0 to 90:10 hexane:ethyl acetate. Colorless oil. Yield: 194 mg (75%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.46 (dd, \( J = 2.5, 0.8 \) Hz, 1H), 8.05 (dd, \( J = 8.4, 2.5 \) Hz, 1H), 7.27 (dd, \( J = 8.4, 0.7 \) Hz, 1H), 6.82 (s, 1H), 2.859 (q, \( J = 7.4 \) Hz, 2H), 2.853 (q, \( J = 7.4 \) Hz, 2H), 1.30 (t, \( J = 7.4 \) Hz, 3H), 1.22 (t, \( J = 7.3 \) Hz, 3H). \(^{13}C \) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 150.3, 149.2, 138.5, 136.9, 131.4, 128.1, 123.5, 128.3, 27.8, 14.9, 14.1. HRMS (+ESI-QTOF) \( m/z \): [M+H]\(^+\) calcd for C\(_{11}\)H\(_{16}\)ClNS\(_2\)\(^+\) 263.0326; found: 263.0323.

\( 1,1 \)-bis(ethylthio)-4-phenyl-1,3-butadiene (4w) Eluent: 100:0 to 90:10 hexane:ethyl acetate. Colorless oil. Yield: 143 mg (57%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.48 – 7.39 (m, 2H), 7.43 (dd, \( J = 15.7, 10.6 \) Hz, 1H), 7.36 – 7.30 (m, 2H), 7.26 – 7.21 (m, 1H), 6.76 (d, \( J = 10.6 \) Hz,
1H), 6.59 (d, J = 15.7 Hz, 1H), 2.843 (q, J = 7.4 Hz, 2H), 2.832 (q, J = 7.4 Hz, 2H), 1.29 (t, J = 7.5 Hz, 3H), 1.27 (t, J = 7.2 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 137.6, 136.2, 133.1, 132.8, 128.8, 127.8, 126.7, 126.0, 28.2, 27.4, 15.2, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C14H19S2+: 251.0923; found: 251.0919.

1,1-bis(ethylthio)-4,8-dimethylnona-1,7-diene (4x) Eluent: 100:0 to 99:1 hexane:ethyl acetate. Colorless oil. Yield: 185 mg (68%). 1H NMR (600 MHz, CDCl3) δ 6.17 (t, J = 7.4 Hz, 1H), 5.09 (tp, J = 7.1, 1.5 Hz, 1H), 2.76 (q, J = 7.3 Hz, 2H), 2.70 (q, J = 7.3 Hz, 2H), 2.35 (ddd, J = 14.4, 7.1, 5.8 Hz, 1H), 2.23 (dt, J = 14.6, 7.6 Hz, 1H), 2.05 – 1.92 (m, 2H), 1.68 (d, J = 1.4 Hz, 3H), 1.60 (d, J = 1.3 Hz, 3H), 1.55 (tt, J = 14.4, 6.4 Hz, 1H), 1.35 (ddt, J = 13.4, 9.5, 6.1 Hz, 1H), 1.22 (dt, J = 7.3 Hz, 3H), 1.20 (dt, J = 7.3 Hz, 3H), 1.20 – 1.14 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H). 13C NMR (151 MHz, CDCl3) δ 140.1, 131.4, 129.5, 124.9, 38.0, 36.9, 33.2, 27.1, 27.0, 25.9, 25.8, 19.8, 17.8, 15.2, 14.5. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C15H29S2+: 273.1705; found: 273.1700.

References


Appendix

S,S'-diethyl phenylketenedithioacetal (4a) $^1$H NMR (400 MHz, CDCl$_3$)

S,S'-diethyl phenylketenedithioacetal (4a) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-fluorophenyl)ketenedithioacetal (4b) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-fluorophenyl)ketenedithioacetal (4b) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-fluorophenyl)ketenedithioacetal (4b) $^{19}$F NMR (376 MHz, CDCl$_3$)

$S,S'$-diethyl (4-chlorophenyl)ketenedithioacetal (4c) $^1$H NMR (400 MHz, CDCl$_3$)
S,S'-diethyl (4-chlorophenyl)ketenedithioacetal (4c) $^{13}$C NMR (101 MHz, CDCl$_3$)

$\text{Cl}$  $\text{S}$  $\text{S}$

S,S'-diisopropyl (4-chlorophenyl)ketenedithioacetal (4d) $^1$H NMR (400 MHz, CDCl$_3$)

$\text{Cl}$  $\text{S}$  $\text{S}$
$S,S'$-diisopropyl (4-chlorophenyl)ketenedithioacetal (4d) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-diphenyl (4-chlorophenyl)ketenedithioacetal (4e) $^1$H NMR (400 MHz, CDCl$_3$)
$S,S'$-diphenyl (4-chlorophenyl)ketenedithioacetal (4e) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-diethyl (4-bromophenyl)ketenedithioacetal (4f) $^1$H NMR (400 MHz, CDCl$_3$)
$S,S'$-diethyl (4-bromophenyl)ketenedithioacetal (4f) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-diethyl (4-(trifluoromethyl)phenyl)ketenedithioacetal (4g) $^1$H NMR (400 MHz, CDCl$_3$)
$S,S'$-diethyl (4-(trifluoromethyl)phenyl)ketenedithioacetal (4g) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-diethyl (4-(trifluoromethyl)phenyl)ketenedithioacetal (4g) $^{19}$F NMR (376 MHz, CDCl$_3$)
$S,S'$-diethyl (4-nitrophenyl)ketenedithioacetal (4h) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-nitrophenyl)ketenedithioacetal (4h) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-cyanophenyl)ketenedithioacetal (4i) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-cyanophenyl)ketenedithioacetal (4i) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-tolyl)ketenedithioacetal (4j) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-tolyl)ketenedithioacetal (4j) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-methoxyphenyl)ketenedithioacetal (4k) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-methoxyphenyl)ketenedithioacetal (4k) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-(methylthio)phenyl)ketenedithioacetal (4l) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (4-(methylthio)phenyl)ketenedithioacetal (4l) $^{13}$C NMR (101 MHz, CDCl$_3$)
$\text{S,S'}$-dimethyl (4-((difluoromethoxy)phenyl)ketenedithioacetal (4m) $^1$H NMR (400 MHz, CDCl$_3$)

$\text{S,S'}$-dimethyl (4-((difluoromethoxy)phenyl)ketenedithioacetal (4m) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-dimethyl (4-(difluoromethoxy)phenyl)ketenedithioacetal (4m) $^{19}$F NMR (376 MHz, CDCl$_3$)

$S,S'$-dimethyl (4-(trifluoromethoxy)phenyl)ketenedithioacetal (4n) $^1$H NMR (400 MHz, CDCl$_3$)
$S,S'$-dimethyl (4-(trifluoromethoxy)phenyl)ketenedithioacetal (4n) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-dimethyl (4-(trifluoromethoxy)phenyl)ketenedithioacetal (4n) $^{19}$F NMR (376 MHz, CDCl$_3$)
$\text{S, S'}$-diethyl (4-(trifluoromethylthio)phenyl)ketenedithioacetal (4o) $^1\text{H}$ NMR (400 MHz, CDCl$_3$)

$\text{S, S'}$-diethyl (4-(trifluoromethylthio)phenyl)ketenedithioacetal (4o) $^{13}\text{C}$ NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (4-(trifluoromethylthio)phenyl)ketenedithioacetal (4o) $^{19}$F NMR (376 MHz, CDCl$_3$)

![Diagram of $S,S'$-diethyl (4-(trifluoromethylthio)phenyl)ketenedithioacetal (4o)]

$S,S'$-diethyl (4-(dimethylamino)phenyl)ketenedithioacetal (4p) $^1$H NMR (400 MHz, CDCl$_3$)

![Diagram of $S,S'$-diethyl (4-(dimethylamino)phenyl)ketenedithioacetal (4p)]
$S, S'$-diethyl (4-(dimethylamino)phenyl)ketenedithioacetal (4p) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S, S'$-diethyl (3,4-methylenedioxyphenyl)ketenedithioacetal (4q) $^1$H NMR (400 MHz, CDCl$_3$)
$S,S'$-diethyl (3,4-methylenedioxyphenyl)ketenedithioacetal (4q) $^{13}$C NMR (101 MHz, CDCl$_3$)

![NMR spectrum of 4q](image)

$S,S'$-diethyl (3,4-(difluoromethylenedioxy)phenyl)ketenedithioacetal (4r) $^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum of 4r](image)
$S,S'$-diethyl (3,4-(difluoromethylenedioxy)phenyl)ketenedithioacetal (4r) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S,S'$-diethyl (3,4-(difluoromethylenedioxy)phenyl)ketenedithioacetal (4r) $^{19}$F NMR (376 MHz, CDCl$_3$)
$S,S'$-dimethyl (naphth-1-yl)ketenedithioacetal (4s) $^1$H NMR (400 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-dimethyl (naphth-1-yl)ketenedithioacetal (4s) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
$\textit{S,S'}$-diethyl thiophen-2-ylketenedithioacetal (4t) $^1$H NMR (400 MHz, CDCl$_3$)

$\textit{S,S'}$-diethyl thiophen-2-ylketenedithioacetal (4t) $^{13}$C NMR (101 MHz, CDCl$_3$)
$S,S'$-diethyl (1-methylindol-3-yl)ketenedithioacetal (4u) $^1$H NMR (400 MHz, CDCl$_3$)

$S,S'$-diethyl (1-methylindol-3-yl)ketenedithioacetal (4u) $^{13}$C NMR (101 MHz, CDCl$_3$)
\[ S,S'-\text{diethyl (2-chloropyridin-5-yl)ketenedithioacetal (4v)} \] $^1$H NMR (400 MHz, CDCl$_3$)

\[ S,S'-\text{diethyl (2-chloropyridin-5-yl)ketenedithioacetal (4v)} \] $^{13}$C NMR (101 MHz, CDCl$_3$)
1,1-bis(ethylthio)-4-phenyl-1,3-butadiene (4w) $^1$H NMR (400 MHz, CDCl$_3$)

1,1-bis(ethylthio)-4-phenyl-1,3-butadiene (4w) $^{13}$C NMR (101 MHz, CDCl$_3$)
1,1-bis(ethylthio)-4-phenyl-1,3-butadiene (4w) HSQC (400 MHz, CDCl₃)

1,1-bis(ethylthio)-4-phenyl-1,3-butadiene (4w) COSY (400 MHz, CDCl₃)
1,1-bis(ethylthio)-4,8-dimethylnona-1,7-diene (4x) $^1$H NMR (400 MHz, CDCl$_3$)

1,1-bis(ethylthio)-4,8-dimethylnona-1,7-diene (4x) $^{13}$C NMR (101 MHz, CDCl$_3$)
1,1-bis(ethylthio)-4,8-dimethylnona-1,7-diene (4x) HSQC (400 MHz, CDCl₃)

1,1-bis(ethylthio)-4,8-dimethylnona-1,7-diene (4x) COSY (400 MHz, CDCl₃) (diagonal-suppressed)
CHAPTER 8. ONE-POT SYNTHESIS OF 5-ALKYLTHTHIO-1,2,4-THIADIAZOLES FROM AMIDINES AND CARBON DISULFIDE

James S. Klimavicz\textsuperscript{1,2,*} and Joel R. Coats\textsuperscript{1}

\textsuperscript{1}Department of Entomology, Iowa State University, Ames, IA, 50011
\textsuperscript{2}Department of Chemistry, Iowa State University, Ames, IA, 50011

Modified from a manuscript for submission to The Journal of Organic Chemistry.

Abstract

\[
\begin{align*}
\text{R} - \text{NH}_2 + \text{CS}_2 + \text{R'X} + \text{DBU} & \rightarrow \text{R} - \text{N}=\text{S} - \text{S} - \text{R'} \\
\text{MeCN, 22 °C, 2-24 hours} & 
\end{align*}
\]

- Mild, one-pot reaction
- Tolerant of air and water
- Tolerant of functional groups
- 35 Examples
- 43-74% Yield
- Scalable

1,2,4-Thiadiazoles have received significant attention as promising building blocks for the development for new agrochemicals and therapeutic compounds due to their potential biological activities. However, the development of new synthetic methods for producing 1,2,4-thiadiazoles has been slow, limiting the exploration of these compounds as pharmaceuticals, coordinating ligands, and organic electronic device materials. In particular, thiadiazoles with sulfur-linked substituents have rarely been explored, and current synthetic routes to these compounds often require harsh reaction conditions, toxic reagents, or expensive building blocks.

Herein, we report the synthesis of 5-alkylthio-1,2,4-thiadiazoles using amidines and carbon

\* JSK optimized the reaction and designed, synthesized, purified, and characterized all compounds and wrote the manuscript. JRC assisted in the revision of the manuscript.
disulfide in a one-pot reaction. The reaction is tolerant of many common functional groups, and produces thiaidazoles in moderate to good yields.

**Introduction**

Over the past decade, 1,2,4-thiadiazoles have gained significant attention as potential building block for the development of new biologically active compounds.\(^1\) Figure 1 shows several 1,2,4-thiadiazoles including cefozopran, a cephalosporin antibiotic with improved activity against methicillin-resistant *Staphylococcus aureus*,\(^2\) an inhibitor of the protease cathepsin B,\(^3\) and the agriculturally important fungicide etridiazole.\(^4-5\) The first natural product containing the 1,2,4-thiadiazole structure, dendroine (Figure 8.1) was isolated in 1980;\(^6\) since this time, several other natural 1,2,4-thiadiazole derivatives have been identified.\(^7-8\) Recently, π-conjugated molecules containing a 1,2,4-thiadiazole core have been reported for use in phosphorescent organic light-emitting diodes.\(^9\) Additionally, peptide mimics containing 1,2,4-thiadiazole moieties have been proposed as chelating compounds for Cu\(^{II}\) ions.\(^10\)

As a bioisostere of carboxylic acid esters,\(^11\) the 1,2,4-thiadiazole ring remains an attractive target for the development of new pharmaceutical compounds due to their biological activity. 5-Alkylthio-1,2,4-thiadiazoles (ATTDs) have also be used as versatile intermediates in the synthesis of other 1,2,4-thiadiazoles.\(^12-13\) While synthetic methods for producing 1,2,4-thiadiazoles have been comprehensively documented in several reviews,\(^1,14-17\) the chemical
space surrounding 1,2,4-thiadiazoles remains underexplored compared to other thiadiazoles and oxadiazoles. Methods for producing ATTDs are uncommon in the literature. Dipotassium cyanodithioiminocarbonate can be oxidized with chlorine gas to produce 3-chloro-1,2,4-thiadiazol-5-ylsulfenyl chloride.\textsuperscript{18} Alternatively, refluxing amidines or amidoximes with carbon disulfide, elemental sulfur, and sodium methoxide yields the corresponding 1,2,4-thiadiazole-5-thione,\textsuperscript{19-20} which may then be deprotonated and \( S \)-alkylated (Scheme 8.1a).\textsuperscript{21} Amidines can also be reacted with trichloromethylsulfenyl chloride, producing 5-chloro-1,2,4-thiadiazoles in modest yield, which react with thiourea to give 1,2,4-thiadiazole-5-thiones, or with thiolates to give the 5-thiolated-1,2,4-thiadiazole (Scheme 8.1b).\textsuperscript{22-23}

To our knowledge, Park et al. have published the only synthesis of an ATTD from an amidine, carbon disulfide, and an alkyl halide that does not use a 1,2,4-thiadiazole-5-thione intermediate; in this case, \( S \)-benzyl \( N \)-(iminobenzyl)dithiocarbamate was synthesized from the benzamidine hydrochloride hydrate in the presence of benzyl chloride, three equivalents of cesium carbonate, three equivalents of carbon disulfide, and catalytic tetrabutylammonium
iodide (TBAI) in tetrahydrofuran (THF) for 24 hours. The dithiocarbamate was then isolated and purified in 74% yield, and 5-benzylthio-3-phenyl-1,2,4-thiadiazole was produced in 99% yield after 24 hours at 60 °C with excess tosyl chloride and pyridine in dichloroethane (Scheme 8.1c). For our synthesis of ATTDs to be competitive with this literature method, we prioritized the simplicity of a mild, one-pot reaction, building the heterocyclic ring from amidines, readily accessible from a variety of methods, and carbon disulfide. Furthermore, we also desired to keep the reaction time relatively short under mild conditions, while keeping yields comparable to previous approaches. Here, we describe such a one-pot synthesis, performed at room temperature and in under 24 hours, producing ATTDs in modest to good yields (Scheme 8.1).

**Results and Discussion**

Our first attempt at synthesizing an ATTD used ethyl imidazole-1-carbodithioate (2) as the carbodithioate donor; this and other imidazole-1-carbodithioates have previously been used for the synthesis of carbamates and other thiocarbonyl compounds from heteroatom nucleophiles. Using benzamidine hydrochloride (1a) with anhydrous potassium carbonate present as a base, S-ethyl N-(iminobenzyl)dithiocarbamate (3a) was isolated in 69% yield, which could then be reacted with N-chlorosuccinimide (NCS) to form 5-ethyl-3-phenyl-1,2,4-thiadiazole (4a) in 91% yield (63% yield from 1a) (Scheme 8.2).

![Scheme 8.2. Synthesis of 4a from benzamidine using ethyl imidazole-1-carbodithioate.](image-url)
Although imidazole-1-carbodithioates are useful carbodithioate donors, they decompose in the presence of moisture and air; additionally, the synthesis of a new imidazole-1-carbodithioate would be required for each change to the S-alkyl group. We therefore sought a method that would allow the synthesis of ATTDs from amidines and alkyl halides using a simple combinatorial approach, without an imidazole-1-carbodithioate intermediate. Because amidines are highly nucleophilic, we were optimistic that benzamidine would react with carbon disulfide in the presence of ethyl bromide to produce 3a, and the reaction proceeded in 27% isolated yield in THF. We then explored the possibility of using NCS in the same pot to oxidize 3a to 4a without the need to isolate the (iminobenzyl)dithiocarbamate, and achieved a 31% yield from the amidine.

The reaction was then optimized by exploring different solvents, bases, and oxidizers, as shown in Table 8.1. Since the goal was to perform the oxidation step in the same pot as the formation of 3a, solvents that could tolerate many oxidants were chosen (entries 1 – 6); of the solvents selected, acetonitrile (ACN) gave the highest yield using K$_2$CO$_3$ as the base. When the reaction was performed in methanol (entry 6), the sole isolated product was 5-methoxy-3-phenyl-1,2,4-thiadiazole.

Because it was speculated that the low solubility of K$_2$CO$_3$ in many solvents might limit the formation of the free amidine, DBU was explored as a relatively strong neutral organic base in several (entries 7 – 10), producing higher yields of 4a than potassium carbonate in solvents used, though ACN still produced the best results. Several other bases were also explored, including anhydrous K$_3$PO$_4$, triethylamine, DABCO, and DBN (entries 11 – 14), and DBU remained the best base of those tested. Neither triethylamine nor DABCO produced any 3a as determined by TLC, likely because benzamidine is a stronger base than either of these amines, so
Table 8.1. Optimization of the one-pot, two-step synthesis of 5-ethylthio-3-phenyl-1,2,4-thiadiazole.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time(^a)</th>
<th>Eq. CS(^2)/EtBr(^b)</th>
<th>Base (Eq.)(^c)</th>
<th>Ox. (Eq.)(^d)</th>
<th>Yield(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THF</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>(39)</td>
</tr>
<tr>
<td>3</td>
<td>Diglyme</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>(12)</td>
</tr>
<tr>
<td>4</td>
<td>DMF</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>nd</td>
</tr>
<tr>
<td>5</td>
<td>CHCl(_3)</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>(6)</td>
</tr>
<tr>
<td>6</td>
<td>MeOH</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>K(_2)CO(_3) (2.5)</td>
<td>NCS (1.05)</td>
<td>0(^f)</td>
</tr>
<tr>
<td>7</td>
<td>THF</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBU (2.5)</td>
<td>NCS (1.05)</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>Diglyme</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBU (2.5)</td>
<td>NCS (1.05)</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>CHCl(_3)</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBU (2.5)</td>
<td>NCS (1.05)</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBU (2.5)</td>
<td>NCS (1.05)</td>
<td>51</td>
</tr>
<tr>
<td>11</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBU (2.5)</td>
<td>NCS (1.2)</td>
<td>(21)</td>
</tr>
<tr>
<td>12</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>Et(_3)N (2.1)</td>
<td>NCS (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DABCO (2.1)</td>
<td>NCS (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>ACN</td>
<td>6 h</td>
<td>1.05/1.05</td>
<td>DBN (2.1)</td>
<td>NCS (1.2)</td>
<td>(47)</td>
</tr>
<tr>
<td>15</td>
<td>ACN</td>
<td>6 h</td>
<td>1.2/1.4</td>
<td>DBU (2.5)</td>
<td>NCS (1.05)</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>ACN</td>
<td>6 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.05)</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>ACN</td>
<td>6 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>64</td>
</tr>
<tr>
<td>18</td>
<td>ACN</td>
<td>6 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>(25)</td>
</tr>
<tr>
<td>19</td>
<td>ACN</td>
<td>6 h(^g)</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>43</td>
</tr>
<tr>
<td>20</td>
<td>ACN</td>
<td>4 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>55</td>
</tr>
<tr>
<td>21</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>67</td>
</tr>
<tr>
<td>22</td>
<td>ACN</td>
<td>16 h</td>
<td>2/3</td>
<td>DBU (2.1)</td>
<td>NCS (1.2)</td>
<td>47</td>
</tr>
<tr>
<td>23</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>NBS (1.2)</td>
<td>55</td>
</tr>
<tr>
<td>24</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>PIDA (1.2)</td>
<td>62</td>
</tr>
<tr>
<td>25</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>PIFA (1.2)</td>
<td>65</td>
</tr>
<tr>
<td>26</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>I(_2) (1.2)</td>
<td>59</td>
</tr>
<tr>
<td>27</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>Chloranil (1.2)</td>
<td>(36)</td>
</tr>
<tr>
<td>28</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>H(_2)O(_2) (1.2)</td>
<td>(23)</td>
</tr>
<tr>
<td>29</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>TCICA (0.4)</td>
<td>(43)</td>
</tr>
<tr>
<td>30</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>DBDMH (0.6)</td>
<td>(56)</td>
</tr>
<tr>
<td>31</td>
<td>ACN</td>
<td>16 h</td>
<td>1.2/1.4</td>
<td>DBU (2.1)</td>
<td>Cu(^{2+}) (0.05) + air(^g)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

Optimization reactions were performed using 0.1 mmol of benzamidine hydrochloride in 1 mL of solvent. 
\(^a\) Reaction time prior to addition of oxidant. 
\(^b\) Equivalents of carbon disulfide and ethyl bromide, respectively. 
\(^c\) Base (equivalents). DABCO: 1,4-diazabicyclo[2.2.2]octane; DBN: 1,5-diazabicyclo(4.3.0)non-5-ene. 
\(^d\) Oxidant (equivalents). NBS: N-bromosuccinimide; PIDA: phenyl-iodine(III) diacetate; PIFA: phenylidodine bis(trifluoroacetate); TCICA: trichloroisocyanuric acid; DBDMH: 1,3-dibromo-5,5-dimethyl-hydantoin. 
\(^e\) Yields in parentheses are estimated by GCMS using 4-chlorobenzonitrile as an internal reference, otherwise the isolated yield is given. 
\(^f\) The major product was 5-methoxy-3-phenyl-1,2,4-thiadiazole, isolated in 38% yield. 
\(^g\) Tetrakis(acetonitrile)copper(I) hexafluorophosphate was used as the copper(II) source.
the free amidine was never formed. In many cases, the main product detected by GCMS was 4-chlorobenzonitrile, likely produced by the direct oxidation of the amidine to the nitrile. It should be noted that DBU may also assist in the formation of 3a as a catalyst, given that cyclic amidines are known to readily add to carbon disulfide.\textsuperscript{31}

Entries 15 – 22 explore the effects of changing the reaction time or temperature, as well as the stoichiometric ratios of the base, oxidant, and other reactants; entry 21 shows the best results obtained. The increased amount of carbon disulfide and ethyl bromide was beneficial as diethyl trithiocarbonate was isolated in small amounts as a side product; the base-mediated formation of trithiocarbonates from carbon disulfide and alkyl halides is known in literature.\textsuperscript{32} However, increasing the ratio of carbon disulfide and ethyl bromide further resulted in a decreased yield (entry 22), likely due to the formation of DBU\textcdot CS\textsubscript{2} adducts.\textsuperscript{31} When other common oxidants were used (entries 23 – 30), lower yields of 4a were obtained compared to NCS. PIDA, PIFA, and iodine all provided qualitatively cleaner reactions by TLC which may be of importance in some applications; indeed, hypervalent iodine reagents have previously been shown to be excellent oxidants for the formation of 5-amino-1,2,4-thiadiazoles.\textsuperscript{33} However, the greater cost of these oxidants, combined with their lower yields, did not justify their exploration for the scope of the reaction. We also attempted an oxidation using catalytic copper(II) and air as an oxidant, as similar systems have been used for the synthesis of 1,2,4-oxadiazoles;\textsuperscript{34} however, these oxidations often require heating, and under the conditions of the reaction, the oxidation was inefficient.

After optimizing the conditions, the scope of the reaction was explored using a variety of amidines and alkyl halides (Table 8.2). We were pleased to find that the reaction worked well
with a wide variety of alkyl bromides and iodides. Using ethyl iodide in place of ethyl bromide to form 4a did not result in a significant improvement of the reaction yield. Isopropyl iodide provided moderate yields of 4d, particularly when compared to 4e, which used isobutyl bromide. Both allyl and benzyl bromides resulted in good yields (4g and 4e). ATTDs 4m, 4n, 4s, and 4t were synthesized with heteroatoms present in the primary halide used in the reaction, and the expected products were isolated in good yield.

A variety of amidines were also explored, including benzamidines substituted with either electron-donating (4q, 4r, and 4u) or electron-withdrawing (4j, 4k, 4o, 4p, and 4v) groups; the

<table>
<thead>
<tr>
<th>Table 8.2. Scope and yields of the one-pot synthesis of ATTDs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a, 67% (69%)</td>
</tr>
<tr>
<td>4h, 70%</td>
</tr>
<tr>
<td>4n, 73%</td>
</tr>
<tr>
<td>4t, 52%</td>
</tr>
<tr>
<td>4z, 56%</td>
</tr>
<tr>
<td>5f, 47% (51%)</td>
</tr>
<tr>
<td>5l, 56%</td>
</tr>
</tbody>
</table>

Reactions performed with 1 mmol amidine hydrochloride and an alkyl bromide except where noted; percentages refer to isolated yields. a Yield in parentheses refers to yield using ethyl iodide instead of ethyl bromide. b Alkyl iodide used instead of alkyl halide. c Performed on a 5-mmol scale. d Hemisulfate salt used. e Yield in parentheses refers to yield in 10% DMPU in ACN was used as the solvent. f Mixture of products obtained; no yield recorded. g Hydrobromide salt used. h Hydroiodide salt used. i Acetate salt used. j Free base used.
yield of the reaction did not appear to depend heavily on the functional groups present on the aromatic ring. While anhydrous amidines were typically used, 4p was synthesized from the amidine hydrochloride dihydrate without any issues. Several heteroarylamidines also provided good yields (4w, 4x, and 4y), though the yields (51 – 62%) were lower than most of those obtained for substituted benzamidines.

Given the success with aryl amidines, we then replaced the aromatic ring with an alkyl group (5a, 5b, and 5c), which did not significantly reduce the yield of the reaction. When ethyl 2-amidinoacetate or malonamamidine were used as the starting amidines, the resultant products (5d and 5e, respectively) were obtained in good yields (Table 8.2). Based on NMR data, these products assume the 3-methylidene-1,2,4-thiadiazoline tautomeric form in both chloroform and DMSO, as opposed to the more frequently observed thiadiazole. We then explored the use of the related compounds O-methylisouronium hemisulfate, S-methylisothiouronium hemisulfate, and N,N-dimethyguanadinium hemisulfate as starting materials. We were pleased when we obtained products 5f and 5i in modest yields. Given the low observed solubility of these hemisulfate salts in acetonitrile, modifying the reaction solvent to 10% N,N'-dimethylpropyleneurea (DMPU) in ACN improved yields slightly. Unfortunately, when S-methylisothiouronium hemisulfate was used, we obtained a roughly stoichiometric mix of 5-ethylthio-3-methylthio-1,2,4-thiadiazole (5g), 3,5-bis(methylthio)-1,2,4-thiadiazole (5g'), 3,5-bis(ethylthio)-1,2,4-thiadiazole (5h), and 3-ethylthio-5-methylthio-1,2,4-thiadiazole (5h'), suggesting that in the corresponding intermediate S,S'-dialkly (dithiocarboxy)isothiourea 3b, the alkylthio groups were easily scrambled (Scheme 8.3); these closely related thiadiazoles could not be separated by column chromatography. Using S-ethylisothiouronium bromide, 5h could be obtained in moderate yield as scrambling no longer led to different products. Other substituted guanidines were used, namely, an N-benzylpiperazine
derivative, and $N^\alpha$-benzoyl-$L$-arginine ethyl ester hydrochloride which produced the expected products ($5j$ and $5k$) in acceptable yields.

Unfortunately, the simplest amidine salt used, formamidine acetate, did not produce any of the expected product $5l$; this is not particularly surprising given that 1,2,4-thiadiazoles lacking 3- and 5-substituents are very sensitive to acids and bases and oxidizing and reducing agents.\textsuperscript{15} Likewise, the use of trifluoroacetamidine did not provide the desired product $5m$, perhaps because of the lowered nucleophilicity of the parent amidine resulting from the strongly electron-withdrawing trifluoromethyl group.

Interestingly, the use of methyl bromoacetate as the alkyl halide did not give the expected ATTD, and instead produced the substituted imidazole $6$ when starting from 4-chlorobenzamidine in 45% yield (calculated from methyl bromoacetate as the limiting reagent). Previous work suggests that the formation of the imidazole occurs through a dialkylated $N$-($\alpha$-aminobenzylidene) dithiocarbamate, which is oxidized to the thiazadiazolium $7$, followed by base-mediated ring-opening and a [4+2] electrocyclization to produce the 2H-1,3,5-thiadiazine $8$, and finally base-mediated desulfurization, as outlined in Scheme 8.4.\textsuperscript{35}
We then explored whether the scope of the reaction could be broadened through the use of other electrophiles (Scheme 8.5). The conjugate addition of thiolates to Michael acceptors is well-established,\textsuperscript{36-38} and we therefore explored the use of methyl acrylate and methyl vinyl ketone in lieu of an alkyl halide. The yields of the ester 9a and ketone 9b were lower than those obtained when using an alkyl halide; however, the reaction conditions were not developed with the thiol-ene click reaction in mind, and we expect that future optimization would improve these yields. 1,2-Epoxybutane also reacted readily with the 4-chlorobenzamidine-carbon disulfide adduct to form 2-hydroxybut-1-yl compound 9c in moderate yield.

Because 5-methoxy-3-phenyl-1,2,4-thiadiazole (10a) was produced during an optimization trial in methanol (Table 8.1, entry 6), we explored the feasibility of using a
modification of the ATTD synthesis to produce other 5-alkoxy-1,2,4-thiadiazoles using the reaction conditions optimized for ATTDs. By using DBU as the base instead of potassium carbonate, and changing the solvent to methanol the reaction of 4-chlorobenzamidine led to the production of 5-methoxy-3-(4-chlorophenyl)-1,2,4-thiadiazole (10b), as well as a small quantity of 4,6-bis(4-chlorophenyl)-2-methoxy-1,3,5-triazine (11b). Table 8.3 shows the yields of 10 and 11 from several different benzamidines. Because the formation of 11 does not require the use of an oxidant, the triazine could be obtained in modest yields from amidine 1, carbon disulfide, ethyl bromide, and DBU by simply heating the reaction to reflux to effect the elimination of ethanethiol and ammonia. Alternatively, using NCS was effective for forming 5-methoxy-1,2,4-thiadiazoles 10, and Scheme 6 shows the synthesis of 10 and 11 from 1. Unfortunately, the production of 10 was frequently accompanied by some of the triazine 11, which, in the examples provided, had similar retention factors to 10, complicating purification efforts. Additionally, the
yields obtained for these 5-alkoxy-1,2,4-thiadiazoles were substantially lower than those for ATTDs, suggesting that optimization of this reaction is necessary before this method becomes attractive for the synthesis of these compounds.

Scheme 8.6 shows the proposed route to the products 10 and 11 from the corresponding amidine. Briefly, the \( N,N' \)-bis(iminomethyl)thiourea 12 is produced by the addition of an equivalent of amidine to 3, accompanied by the loss of ethanethiol. In an intramolecular addition, similar to that of the Pinner triazole synthesis,\(^{39-40} \) 12 cyclizes to form 1,3,5-triazine-2(5\( H \))-thione 13 after the loss of ammonia. In the presence of ethyl bromide and DBU, 13 is S-alkylated to form 14, which, in the presence of DBU and methanol, undergoes nucleophilic displacement to yield the methoxy-substituted triazine 11. Compound 14 was not detected when the reaction was run in methanol, suggesting that the substitution reaction occurs rapidly; however, when the reaction was performed in acetonitrile at reflux instead of methanol, 14 was isolated in 34% yield. When we attempted to synthesize higher analogs of 10 by performing the reaction in ethanol or isopropanol in lieu of methanol, no thiadiazole or triazine was detected. However, by
simply changing the solvent from neat ethanol to a 1:1 mix of acetonitrile and ethanol allowed us to produce the expected 5-ethoxy-3-phenyl-1,2,4-thiadiazole (15) in 24% yield (Scheme 8.7).

**Conclusions**

In summary, we have developed a versatile synthesis of 5-alkylthio-1,2,4-thiadiazoles from amidines and alkyl halides using carbon disulfide as a carbon and sulfur source, and \( N \)-chlorosuccinimide as an easily-accessible oxidant. The reaction works with alkyl, aryl, and heteroarylamidines, as well as \( N \)-substituted guanidines and \( O \)-substituted isoureas, while using \( S \)-substituted isothioureas results in a mixture of products due to scrambling of the alkylthio groups. Although this synthesis of 5-alkylthio-1,2,4-thiadiazoles is somewhat narrowed when using alkyl halides on the basis of steric demands other nucleophiles like methyl acrylate and 1,2-epoxybutane also react with the intermediate amidine-carbon disulfide adduct, permitting the formation of diverse thiadiazoles. Additionally, by changing the reaction conditions, several other products could be obtained, including either 5-methoxy-1,2,4-thiadiazoles or 2-methoxy-1,3,5-triazines when performing the reaction in methanol. Given that 1,2,4-thiadiazoles have traditionally been underrepresented in leads for biologically active compounds, we hope that this new, one-pot synthesis of 5-alkylthio-1,2,4-thiadiazoles will improve the availability of these compounds for future exploration.
Acknowledgments

The authors would like to thank the Dr. Peter Porpiglia and AMVAC Chemical Corporation for their support in funding this research, and Dr. George Kraus for his review of this paper. We also wish to thank Drs. Sarah Cady, Shu Xu, and Kamel Harrata for training and assistance in the Iowa State University Chemical Instrumentation Facility.

Experimental

General Information

All solvents were purchased from Fisher Scientific and used as received. Potassium carbonate was ground in a mortar and pestle and oven-dried at 250 °C for 24 hours before use. All amidine salts were purchased from TCI, Alfa Aesar, Oakwood Chemicals, Chem-Impex, or Maybridge and were used as received. Reaction products were visualized via TLC under UV light or by staining with KMnO4 or cerium ammonium molybdenate stain. 5-Alkylthio-1,2,4-thiadiazoles were purified on a Buchi Pure C-810 Flash chromatography system using HPLC grade solvents on 12 g or 25 g FlashPure silica cartridges, except where noted. The characterization of all compounds was performed at the Iowa State University Chemical Instrumentation Facility. NMR spectra were obtained using Avance NEO 400 MHz and Avance III 600 MHz spectrometers. Chemical shifts are reported in ppm relative to the residual solvent peak (CDCl3: 7.26 ppm for 1H and 77.16 ppm for 13C; DMSO-d6: 2.50 for 1H and 39.52 ppm for 13C). Coupling constants are reported in Hz. HRMS analysis was performed using positive ion mode mass spectra on an Agilent QTOF 6540 mass spectrometer. Accurate mass measurement was achieved by constantly infusing a calibrant (masses: 121.0508 and 922.0098). Melting points are uncorrected were obtained on Stuart SMP30 melting point apparatus using a temperature ramp rate of not greater than 5 °C min⁻¹.
**Ethyl 1-imidazolecarbodithioate (2)** Modifying a literature procedure, sodium hydride (60% dispersion in oil, 0.504 g, 1.05 eq) was suspended in dry THF (50 mL) under an argon atmosphere, and imidazole (1.362 g, 20 mmol) was added portionwise over five minutes at 0 ºC. The suspension was stirred at this temperature for 15 minutes, during which time a heavy precipitate formed. Carbon disulfide (1.675 g, 1.1 eq) was added over 3 minutes at 0 ºC, and the solution became clear and deep orange. After 30 min at 0ºC, bromoethane (2.397 g, 1.1 eq) was added in one portion, and the reaction was warmed to room temperature and stirred for 1 hour. The solution was then reduced under vacuum to a volume of approximate 20 mL, and ethyl acetate (50 mL) and water (20 mL) were added. The aqueous layer was removed, and the organic layer was washed again with water (20 mL) and brine (20 mL), and then dried over anhydrous magnesium sulfate. The solvent was removed, and the crude material was purified by flash chromatography (80:20 hexane:ethyl acetate) to yield a bright yellow oil (2.69 g, 78%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.47 (t, $J$ = 1.0 Hz, 1H), 7.77 (t, $J$ = 1.5 Hz, 1H), 7.09 (dd, $J$ = 1.8, 0.9 Hz, 1H), 3.39 (q, $J$ = 7.4 Hz, 2H), 1.43 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 198.2, 135.7, 131.4, 117.8, 31.4, 12.6.

**S-Ethyl-N-(a-iminobenzyl)dithiocarbamate (3a)** From 2: Benzamidine hydrochloride (157mg, 1 mmol) and powdered anhydrous potassium carbonate (346 mg, 2.5 eq) were added to acetonitrile (5 mL), followed by the addition of 2 (181 mg, 1.05 eq). The reaction was heated to 50 ºC for 18 hours, and then cooled to 22 ºC. the reaction was then poured into water (25 mL), and extracted twice with ethyl acetate (20 mL). The combined organic layers were washed twice with water (20 mL) and brine (20 mL), and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the crude deep orange liquid was purified by column
chromatography (eluent: 90:10 to 60:40 hexane:ethyl acetate) to yield the title compound as a
viscous orange liquid (190 mg, 85% yield).

From carbon disulfide and bromoethane: Benzamidine hydrochloride (157 mg, 1 mmol)
and DBU (167 mg, 1.1 mmol) were dissolved in acetonitrile (10 mL) at 22 ºC, and carbon
disulfide (114 mg, 1.1 mmol) and bromoethane (131 mg) were added in one portion. The
reaction was allowed to stir 18 hours, and the reaction was then worked up as above. \(^1\)H NMR
(400 MHz, CDCl\(_3\)) \(\delta\) 11.4 (broad s, 1H), 7.97 – 7.90 (m, 2H), 7.62 – 7.54 (m, 1H), 7.53 – 7.46
(m, 2H), 7.0 (broad s, 1H), 3.21 (q, \(J = 7.4\) Hz, 2H), 1.38 (t, \(J = 7.4\) Hz, 3H). \(^1\)H NMR (400
MHz, DMSO-\(d_6\)) \(\delta\) 10.59 (broad s, 1H), 9.68 (broad s, 1H), 8.06 – 7.98 (m, 2H), 7.68– 7.59 (m,
1H), 7.59 – 7.51 (m, 1H), 3.10 (q, \(J = 7.3\) Hz, 2H), 1.27 (t, \(J = 7.4\) Hz, 3H). \(^1\)H NMR (400
MHz, CDCl\(_3\)) \(\delta\) 217.4, 161.9, 134.0, 132.9, 129.0, 127.7, 29.8, 13.4. \(^1\)C NMR (101 MHz,
CDCl\(_3\)) \(\delta\) 212.8, 161.7, 133.5, 132.7, 128.7, 128.2, 28.8, 14.0. HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for
C\(_{10}\)H\(_{13}\)N\(_2\)S\(_2\)\(^2+\) 225.0515; found: 225.0512.

\(S\)-Ethyl-N-(\(\alpha\)-imino-4-chlorobenzyl)dithiocarbamate (3b) Collected as an impurity
during the synthesis of 14. Yellow-orange crystalline solid. mp 116–118 ºC \(^1\)H NMR (600 MHz,
CDCl\(_3\)) \(\delta\) 11.3 (broad s, 1H), 7.88 – 7.82 (m, 2H), 7.46 – 7.41 (m, 2H), 6.8 (broad s, 1H), 3.18
(q, \(J = 7.4\) Hz, 2H), 1.36 (t, \(J = 7.4\) Hz, 3H). \(^1\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 218.5, 160.4, 139.1,
133.0, 129.3, 129.0, 29.8, 13.5. HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for C\(_{10}\)H\(_{12}\)ClN\(_2\)S\(_2\)\(^2+\)
259.0125; found: 259.0127.

**General procedure for the synthesis of 5-alkylthio-1,2,4-thiadiazoles (GP1)**

Amidine hydrochloride (1.0 mmol) and DBU (312 mg, 2.05 mmol) were added to
acetonitrile (10 mL), followed by the addition of carbon disulfide (84 mg, 1.1 mmol) and alkyl
halide (1.2 mmol), and the reaction was stirred at 40 ºC for 18 hours. The deep yellow-to-red
reaction mixture was then cooled to 0 ºC, and NCS (147 mg, 1.1 eq) was added in a single
portion. The reaction was stirred at 22 °C and 1,2,4-thiadiazole formation was monitored by TLC, and upon consumption of the S-alkyl (iminomethyl)dithiocarbamate (typically 15–60 minutes), excess NCS was quenched by the addition of 1 M sodium thiosulfate solution (2 mL). The reaction was poured into water (15 mL), and the biphasic mixture was extracted with hexane (2 x 10 mL). The organic layer was washed with water (2 x 10 mL), then 1 M hydrochloric acid (10 mL), 1 M sodium hydroxide (10 mL), and brine (10 mL), and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the crude product was purified by flash chromatography on silica using a gradient of 100:0 to 90:10 hexane:ethyl acetate as an eluent.

5-Ethylthio-3-phenyl-1,2,4-thiadiazole\textsuperscript{42} (4a) From 3a: To acetonitrile (5 mL) was added 3aa (224 mg, 1 mmol) and DBU (160 mg, 1.05 eq). The solution was cooled to 0 °C, and NCS (147 mg, 1.1 eq) was added. After 30 minutes, the reaction was worked up as in GP1.

From 1a, Method A: Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and bromoethane (131 mg). Colorless liquid that solidifies to a white, crystalline solid upon cooling (163 mg, 73% yield). From 1a, Method B: Iodoethane (187 mg) used instead of bromoethane. Yield: 165 mg (75%). mp 37–39 °C (lit.\textsuperscript{42} 37–38 °C). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.31 – 8.25 (m, 2H), 7.51 – 7.43 (m, 3H), 3.34 (q, \(J = 7.4\) Hz, 2H), 1.54 (t, \(J = 7.4\) Hz, 3H). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 187.6, 172.6, 132.7, 130.5, 128.8, 128.4, 28.8, 14.4. HRMS (+ESI-QTOF) \(m/z\): [M+H]\textsuperscript{+} calcd for C\textsubscript{10}H\textsubscript{11}N\textsubscript{2}S\textsubscript{2} 223.0358; found: 223.0357.

5-Methylthio-3-phenyl-1,2,4-thiadiazole\textsuperscript{43} (4b) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and iodomethane (170 mg). Eluent: 100:0 to 90:10 hexane:ethyl acetate. White, crystalline solid (153 mg, 69% yield). mp 81–82 °C (lit.\textsuperscript{43} 76 °C). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.31 – 8.25 (m, 2H), 7.50 – 7.44 (m, 3H), 2.80 (s, 3H). \textsuperscript{13}C NMR
(101 MHz, CDCl$_3$) $\delta$ 188.7, 172.7, 132.6, 130.5, 128.8, 128.4, 16.7. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_9$H$_9$N$_2$S$_2^+$ 209.0202; found: 209.0198.

3-Phenyl-5-propylthio-1,2,4-thiadiazole (4c) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and 1-bromopropane (148 mg). Colorless liquid (162 mg, 68% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.32 – 8.22 (m, 2H), 7.54 – 7.41 (m, 3H), 3.30 (t, $J$ = 7.2 Hz, 2H), 1.91 (h, $J$ = 7.3 Hz, 2H), 1.12 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 188.7, 172.7, 132.6, 130.5, 128.8, 128.4, 16.7. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_9$H$_9$N$_2$S$_2^+$ 209.0202; found: 209.0198.

5-Isopropylthio-3-phenyl-1,2,4-thiadiazole (4d) Synthesized according to GP1 from benzamide hydrochloride (157 mg) and 2-iodopropane (204 mg). Colorless oil (137 g, 51%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.31 – 8.24 (m, 2H), 7.50 – 7.44 (m, 3H), 4.00 (hept, $J$ = 6.8 Hz, 1H), 1.56 (d, $J$ = 6.8 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 187.0, 172.5, 132.7, 130.5, 128.8, 128.4, 40.5, 23.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{13}$N$_2$S$_2^+$ 237.0515; found: 237.0514.

5-Isobutylthio-3-phenyl-1,2,4-thiadiazole (4e) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and 1-bromo-2-methylpropane (164 mg). Colorless liquid (169 mg, 44% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.32 – 8.22 (m, 1H), 7.54 – 7.41 (m, 2H), 3.22 (d, $J$ = 6.8 Hz, 1H), 2.14 (dh, $J$ = 6.8, 6.6 Hz, 1H), 1.12 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 188.3, 172.5, 132.7, 130.5, 128.8, 128.4, 43.1, 28.7, 22.1. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{15}$N$_2$S$_2^+$ 251.0671; found: 251.0673.

5-(Cyclopropylmethylthio)-3-phenyl-1,2,4-thiadiazole (4f) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and cyclopropylmethyl bromide (162 mg). Colorless liquid (169 mg, 68% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.33 – 8.22 (m, 1H), 7.52 –
7.41 (m, 2H), 3.29 (d, $J = 7.3$ Hz, 1H), 1.29 (ttt, $J = 8.0, 7.3, 4.7$ Hz 1H), 0.79 – 0.62 (m, 1H), 0.48 – 0.37 (m, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 187.8, 172.5, 132.7, 130.5, 128.8, 128.4, 40.4, 10.5, 6.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{13}$N$_2$S$_2^+$ 249.0515; found: 249.0514.

5-Allylthio-3-phenyl-1,2,4-thiadiazole$^{43}$ (4g) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and allyl bromide (145 mg). Colorless liquid (173 mg, 74% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.32 – 8.22 (m, 2H), 7.51 – 7.41 (m, 3H), 6.02 (ddt, $J = 16.9, 10.0, 6.9$ Hz, 1H), 5.45 (dq, $J = 16.9, 1.3$ Hz, 1H), 5.28 (dq, $J = 10.0, 1.0$ Hz, 1H), 3.97 (dt, $J = 6.9, 1.2$ Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 186.7, 172.4, 132.6, 131.7, 130.5, 128.8, 128.4, 120.1, 37.0. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{11}$N$_2$S$_2^+$ 235.0358; found: 235.0361.

5-Benzylthio-3-phenyl-1,2,4-thiadiazole$^{12}$ (4h) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and benzyl bromide (205 mg). Viscous, colorless oil (198 mg, 70% yield); lit.$^{12}$ white solid; no mp given. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.33 – 8.26 (m, 2H), 7.53 – 7.43 (m, 5H), 7.39 – 7.28 (m, 3H), 4.57 (s, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 186.8, 172.3, 135.6, 132.6, 130.5, 129.2, 129.0, 128.8, 128.4, 38.6. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{15}$H$_{13}$N$_2$S$_2^+$ 285.0515; found: 285.0515.

5-Citronellylthio-3-phenyl-1,2,4-thiadiazole (4i) Synthesized according to GP1 from benzamidine hydrochloride (157 mg) and racemic citronellyl bromide (263 mg). Eluent: 100:0 to 93:7 hexane:ethyl acetate. Colorless oil (201 mg, 60% yield). $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.31 – 8.24 (m, 2H), 7.50 – 7.43 (m, 3H), 5.10 (tp, $J = 7.1, 1.4$ Hz, 1H), 3.36 (ddd, $J = 12.6, 9.6, 5.3$ Hz, 1H), 3.29 (ddd, $J = 12.6, 9.2, 6.3$ Hz, 1H), 2.04 (dp, $J = 14.6, 7.2$ Hz, 2H), 1.99 (dp, $J = 14.6, 7.2$ Hz, 2H), 1.89 (ddddd, $J = 12.7, 9.2, 6.2, 4.8$ Hz, 1H), 1.75 – 1.62 (m, 5H), 1.60 (d, $J = 1.3$ Hz,
5-Ethylthio-3-(4-fluorophenyl)-1,2,4-thiadiazole (4j) Synthesized according to the general procedure from 4-fluorobenzamidine hydrochloride (175 mg) and bromoethane (131 mg). White, crystalline solid (177 mg, 74% yield). mp 35–36 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.29 – 8.24 (m, \(J_{HF} = 11.4\), 2H), 7.17 – 7.11 (m, \(J_{HF} = 8.8\), 2H), 3.32 (q, \(J = 7.4\) Hz, 2H), 1.53 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 187.9, 171.5, 164.3 (d, \(J_{CF} = 250.5\) Hz), 130.5 (d, \(J_{CF} = 8.7\) Hz), 129.0 (d, \(J_{CF} = 3.2\) Hz), 115.8 (d, \(J_{CF} = 21.9\) Hz), 28.9, 14.4. \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -110.06 (tt, \(J = 8.6\), 5.3 Hz). HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for C\(_{10}\)H\(_{10}\)FN\(_2\)S\(_2^+\) 241.0264; found: 241.0260.

3-(4-Chlorophenyl)-5-ethylthio-1,2,4-thiadiazole (4k) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and bromoethane (131 mg). White crystalline solid (188 mg, 73% yield). mp 44–45 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.24 – 8.17 (m, 2H), 7.48 – 7.40 (m, 2H), 3.33 (q, \(J = 7.4\) Hz, 2H), 1.54 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 188.1, 171.5, 136.6, 131.2, 129.7, 129.0, 28.9, 14.4. HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for C\(_{10}\)H\(_{10}\)ClN\(_2\)S\(_2^+\) 256.9968; found: 256.9970.

3-(4-Chlorophenyl)-5-methylthio-1,2,4-thiadiazole (4l) Performed on 5 mmol scale from 4-chlorobenzamidine hydrochloride (955 mg, 5 mmol) and iodomethane (681 mg, 6.25 mmol, 1.2 eq). Beige semi-crystalline solid, which may be recrystallized from hot heptane to give white needles (846 mg, 70% yield). mp 105–107 ºC (lit.\(^{43}\) 101 ºC). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.24 – 8.17 (m, 2H), 7.46 – 7.40 (m, 2H), 2.79 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\)
189.12, 171.63, 136.61, 131.10, 129.73, 129.02, 16.70. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C_{9}H_{8}ClN_{2}S_{2}^{+} 242.9812; found: 242.9810.

3-(4-Chlorophenyl)-5-(3-chloroprop-1-yl)-1,2,4-thiadiazole (4m) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and 1-bromo-3-chloropropane (236 mg). Pale yellow liquid (224 mg, 73% yield), containing a small amount of 4-chlorobenzonitrile as an impurity. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.23 – 8.17 (m, 1H), 7.49 – 7.38 (m, 1H), 3.72 (t, \(J = 6.1\) Hz, 1H), 3.51 (t, \(J = 6.9\) Hz, 1H), 2.35 (tt, \(J = 6.9, 6.1\) Hz, 1H). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 187.1, 171.4, 136.7, 131.0, 129.7, 129.1, 43.2, 31.7, 31.4. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C\(_{11}\)H\(_{11}\)ClN\(_2\)S\(_{2}\)^{+} 304.9735; found: 304.9734.

5-((2-Bromo-5-methoxybenzyl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (4n) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and 2-bromo-5-methoxybenzyl bromide (420 mg). White platelets (311 mg, 73%). \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.27 – 8.22 (m, 2H), 7.48 (d, \(J = 8.8\) Hz, 1H), 7.46 – 7.43 (m, 2H), 7.18 (d, \(J = 3.0\) Hz, 1H), 6.74 (dd, \(J = 8.8, 3.1\) Hz, 1H), 4.67 (s, 2H), 3.74 (s, 3H). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 186.8, 171.0, 159.0, 136.6, 136.0, 133.8, 131.0, 129.6, 129.0, 116.8, 115.5, 115.0, 55.5, 39.0. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C\(_{16}\)H\(_{13}\)BrClN\(_2\)OS\(_2\)^{+} 426.9336; found: 426.9340.

3-(4-Bromophenyl)-5-ethylthio-1,2,4-thiadiazole (4o) Synthesized according to the general procedure from 4-bromobenzamidine hydrochloride (236 mg) and bromoethane (131 mg). Light tan crystalline solid (223 mg, 74% yield). mp 36–37 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.18 – 8.10 (m, 2H), 7.63 – 7.55 (m, 2H), 3.33 (q, \(J = 7.4\) Hz, 2H), 1.53 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 188.1, 171.5, 132.0, 131.6, 129.9, 125.0, 28.8, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C\(_{10}\)H\(_{10}\)BrN\(_2\)S\(_2\)^{+} 300.9463; found: 300.9464.
5-Ethylthio-3-(4-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (4p) Synthesized according to GP1 from 4-(trifluoromethyl)benzamidine hydrochloride dihydrate (261 mg) and bromoethane (131 mg). Eluent: 100:0 to 95:5 hexane:ethyl acetate. Colorless oil (197 mg, 68% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.42 – 8.35 (m, 2H), 7.75 – 7.68 (m, 2H), 3.35 (q, $J$ = 7.4 Hz, 3H), 1.54 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 188.5, 171.1, 135.7, 132.1 (q, $J$ = 32.5 Hz), 128.7, 125.8 (q, $J$ = 3.9 Hz), 124.1 (q, $J$ = 273.3 Hz), 28.9, 14.4. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -62.8. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{11}$H$_{10}$F$_3$N$_2$S$_2^+$ 291.0232; found: 291.0232.

5-Ethylthio-3-(4-methoxyphenyl)-1,2,4-thiadiazole (4q) Synthesized according to the general procedure from 4-methoxybenzamidine hydrochloride (187 mg) and bromoethane (131 mg). Eluent: 100:0 to 85:15 hexane:ethyl acetate. White crystalline solid (147 mg, 58% yield). mp 54–56 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.25 – 8.17 (m, 2H), 7.01 – 6.93 (m, 2H), 3.87 (s, 3H), 3.32 (q, $J$ = 7.4 Hz, 2H), 1.53 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 187.3, 172.4, 161.5, 130.0, 125.7, 114.1, 55.5, 28.8, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{11}$H$_{13}$N$_2^+$ 253.0464; found: 253.0466.

5-Ethylthio-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4r) Synthesized according to the general procedure from 3-methoxybenzamidine hydrochloride (187 mg) and bromoethane (131 mg). Extracted with ethyl acetate. Eluent: 100:0 to 80:20 hexane:ethyl acetate. Colorless oil (154 mg, 61% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.88 (ddd, $J$ = 7.6, 1.5, 1.0 Hz, 1H), 7.82 (dd, $J$ = 2.7, 1.5 Hz, 1H), 7.37 (dd, $J$ = 8.3, 7.6 Hz, 1H), 7.01 (ddd, $J$ = 8.3, 2.7, 1.0 Hz, 1H), 3.89 (s, 3H), 3.33 (q, $J$ = 7.4 Hz, 2H), 1.53 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 187.6, 172.4, 160.0, 133.9, 129.8, 121.0, 117.0, 113.0, 55.6, 28.8, 14.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{11}$H$_{13}$N$_2$OS$_2^+$ 253.0464; found: 253.0466.
5-((2-Methoxyethyl)thio)-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4s) Synthesized according to the general procedure from 3-methoxybenzamidine hydrochloride (187 mg) and bromoethyl methyl ether (167 mg). Extracted with ethyl acetate. Eluent: 100:0 to 80:20 hexane:ethyl acetate. Colorless oil (186 mg, 66% yield). $^1$H NMR (600 MHz, CDCl$_3$) δ 7.86 (ddd, $J$ = 7.7, 1.5, 1.0 Hz, 2H), 7.80 (dd, $J$ = 2.7, 1.5 Hz, 1H), 7.37 (dd, $J$ = 8.3, 7.7 Hz, 1H), 7.00 (ddd, $J$ = 8.3, 2.7, 1.0 Hz, 1H), 3.88 (s, 3H), 3.79 (t, $J$ = 6.2 Hz, 2H), 3.54 (t, $J$ = 6.2 Hz, 2H), 3.42 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 187.2, 172.1, 159.9, 133.8, 129.8, 121.0, 116.9, 113.0, 70.5, 59.0, 55.5, 34.1. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{12}$H$_{15}$N$_2$O$_3$S$_2^+$ 283.0569; found: 283.0569.

tert-Butyl (2-((3-methoxyphenyl)-1,2,4-thiadiazol-5-yl)thio)ethyl)carbamate (4t)
Synthesized according to the general procedure from 3-methoxybenzamidine hydrochloride (187 mg) and N-Boc-2-bromoethylamine (269 mg). Extracted with ethyl acetate. Eluent: 100:0 to 80:20 hexane:ethyl acetate to give a white, fibrous solid (192 mg, 52% yield). mp 86–87 ºC. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.85 (ddd, $J$ = 7.6, 1.5, 1.0 Hz, 1H), 7.79 (dd, $J$ = 2.7, 1.5 Hz, 1H), 7.36 (t, $J$ = 7.9 Hz, 1H), 7.00 (ddd, $J$ = 8.3, 2.7, 1.0 Hz, 1H), 5.22 (s, 1H), 3.88 (s, 3H), 3.60 (q, $J$ = 6.2 Hz, 2H), 3.47 (t, $J$ = 6.3 Hz, 2H), 1.43 (s, 9H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 186.8, 172.2, 159.9, 155.9, 133.7, 129.8, 121.0, 116.9, 113.1, 79.8, 55.5, 40.2, 34.6, 28.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{16}$H$_{21}$N$_3$O$_3$S$_2^+$ 368.1097; found: 368.1096.

5-Ethylthio-3-(3-toluyl)-1,2,4-thiadiazole (4u) Synthesized according to the general procedure from 3-methylbenzamidine hydrochloride (171 mg) and bromoethane (131 mg).
Eluent: 100:0 to 95:5 hexane:ethyl acetate. Colorless oil (166 mg, 70% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.09 (s, 1H), 8.07 (d, $J$ = 7.6 Hz, 1H), 7.36 (t, $J$ = 7.6 Hz, 1H), 7.27 (d, $J$ = 7.6 Hz, 1H), 3.34 (q, $J$ = 7.4 Hz, 1H), 2.43 (s, 1H), 1.54 (t, $J$ = 7.4 Hz, 2H). $^{13}$C NMR (101 MHz,
CDCl$_3$ $\delta$ 187.6, 172.8, 138.5, 132.6, 131.3, 129.0, 128.7, 125.6, 28.8, 21.6, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{11}$H$_{13}$N$_2$S$_2$ 237.0515; found: 237.0518.

5-Ethylthio-3-(4-nitrophenyl)-1,2,4-thiadiazole (4v) Synthesized according to the general procedure from 4-nitrobenzamidine hydrochloride (202 mg) and bromoethane (131 mg). Eluent: 100:0 to 80:20 hexane:ethyl acetate. Pale yellow prisms (174 mg, 65% yield). mp 103–104 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.49 – 8.41 (m, 1H), 8.36 – 8.28 (m, 1H), 3.36 (q, $J$ = 7.4 Hz, 1H), 1.56 (t, $J$ = 7.4 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 189.0, 170.2, 149.0, 138.0, 129.3, 124.1, 29.0, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{10}$H$_{10}$N$_3$O$_2$S$_2$ 268.0209; found: 268.0207.

5-Ethylthio-3-(pyridin-2-yl)-1,2,4-thiadiazole (4w) Synthesized according to the general procedure from pyridine-2-carboxamidine hydrochloride (158 mg) and bromoethane (131 mg). Extracted with ethyl acetate. Eluent: 100:0 to 20:80 hexane:ethyl acetate. Pale yellow oil (139 mg, 62% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.78 (ddd, $J$ = 4.8, 1.8, 0.9 Hz, 1H), 8.31 (dt, $J$ = 7.9, 1.1 Hz, 1H), 7.83 (td, $J$ = 7.7, 1.8 Hz, 1H), 7.37 (ddd, $J$ = 7.6, 4.8, 1.2 Hz, 1H), 3.33 (q, $J$ = 7.4 Hz, 2H), 1.53 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 189.0, 171.8, 150.5, 150.3, 137.1, 124.8, 123.9, 28.9, 14.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{9}$H$_{10}$N$_3$O$_2$S$_2$ 224.0311; found: 224.0312.

5-Ethylthio-3-(pyridin-4-yl)-1,2,4-thiadiazole (4x) Synthesized according to the general procedure from pyridine-4-carboxamidine hydrochloride (158 mg) and bromoethane (131 mg). Extracted with ethyl acetate. Eluent: 85:15 to 0:100 hexane:ethyl acetate. White crystalline solid (117 mg, 52% yield). mp 49–50 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.74 (d, $J$ = 6.2 Hz, 2H), 8.09 (d, $J$ = 6.2 Hz, 2H), 8.09 (d, $J$ = 6.2 Hz, 2H), 3.34 (q, $J$ = 7.4 Hz, 2H), 1.53 (t, $J$ = 7.4 Hz, 4H). $^{13}$C NMR (101 MHz,
CDCl$_3$ δ 188.9, 170.3, 150.7, 139.2, 122.1, 28.9, 14.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_9$H$_{10}$N$_3$S$_2^+$ 224.0311; found: 224.0314.

**5-Ethylthio-3-(pyrazin-2-yl)-1,2,4-thiadiazole (4y)** Synthesized according to the general procedure from pyrazine-2-carboxamidine hydrochloride (159 mg) and bromoethane (131 mg). Extracted with ethyl acetate. Eluent: 100:0 to 20:80 hexane:ethyl acetate. White crystalline solid (132 mg, 59% yield). mp 60–62 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.53 (d, $J= 1.5$ Hz, 1H), 8.73 (dd, $J= 2.5$, $1.5$ Hz, 1H), 8.66 (d, $J= 2.5$ Hz, 1H), 3.36 (q, $J= 7.4$ Hz, 2H), 1.54 (t, $J= 7.4$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 189.8, 169.5, 146.1, 145.6, 145.4, 144.7, 29.0, 14.2. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_8$H$_9$N$_4$S$_2^+$ 225.0263; found: 225.0263.

**5-Ethylthio-3-methyl-1,2,4-thiadiazole (5a)** Synthesized according to the general procedure from acetamidine hydrochloride (94.5 mg) and bromoethane (131 mg). Eluent: 100:0 to 95:5 hexane:ethyl acetate. Colorless oil with unpleasant odor (89.2 mg, 56% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 3.23 (q, $J= 7.4$ Hz, 2H), 2.61 (s, 3H), 1.48 (t, $J= 7.4$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 187.5, 173.1, 28.7, 19.0, 14.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_5$H$_9$N$_2$S$_2^+$ 161.0202; found: 161.0200.

**3-Cyclopropyl-5-ethylthio-1,2,4-thiadiazole (5b)** Synthesized according to the general procedure from cyclopropanecarboxamidine hydrochloride (121 mg) and bromoethane (131 mg). Eluent: 100:0 to 95:5 hexane:ethyl acetate. Colorless oil. (121 mg, 65% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 3.20 (q, $J= 7.4$ Hz, 2H), 2.26 (tt, $J= 8.2$, 4.9 Hz, 1H), 1.46 (t, $J= 7.4$ Hz, 3H), 1.14 – 1.09 (m, 2H), 1.05 – 1.00 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 187.0, 178.2, 28.6, 14.3, 13.8, 9.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_7$H$_{11}$N$_2$S$_2^+$ 187.0358; found: 187.0356.
3-(2,6-Dichlorobenzyl)-5-ethylthio-1,2,4-thiadiazole (5c) Synthesized according to the general procedure from 2-(2,6-dichlorophenyl)ethanimidamide hydrochloride (240 mg) and bromoethane (131 mg). Eluent: 100:0 to 95:5 hexane:ethyl acetate to yield a pale yellow solid (172 mg, 56%). \( ^1\text{H NMR (400 MHz, CDCl}_3 \) \( \delta \) 7.35 (d, \( J = 8.0 \) Hz, 1H), 7.18 (t, \( J = 8.0 \) Hz, 1H), 4.60 (s, 1H), 3.22 (q, \( J = 7.4 \) Hz, 1H), 1.46 (t, \( J = 7.4 \) Hz, 1H). \( ^{13}\text{C NMR (101 MHz, CDCl}_3 \) \( \delta \) 187.82, 172.40, 136.32, 133.67, 128.87, 128.28, 35.07, 28.76, 14.26. HRMS (+ESI-QTOF) \( m/z: [M+H]^+ \) calcd for C\(_{11}\)H\(_{11}\)Cl\(_2\)N\(_2\)S\(_2^+\) 304.9735; found: 304.9739.

Ethyl 2-(5-ethylthio-1,2,4-thiadiazol-3(2H)-ylidene)acetate (5d) Synthesized according to the general procedure from 2-carbethoxyacetamidine hydrochloride (167 mg) and bromoethane (131 mg). Eluent: 100:0 to 85:15 hexane:ethyl acetate. White crystalline solid (165 mg, 71% yield). mp 128–129 °C. \( ^1\text{H NMR (400 MHz, CDCl}_3 \) \( \delta \) 5.76 (broad s, 2H), 4.36 (q, \( J = 7.1 \) Hz, 2H), 2.99 (q, \( J = 7.4 \) Hz, 2H), 1.46 (t, \( J = 7.4 \) Hz, 3H), 1.40 (t, \( J = 7.1 \) Hz, 3H). \( ^{13}\text{C NMR (101 MHz, CDCl}_3 \) \( \delta \) 173.9, 165.2, 163.2, 109.4, 61.1, 28.1, 14.5, 13.5. HRMS (+ESI-QTOF) \( m/z: [M+H]^+ \) calcd for C\(_8\)H\(_{13}\)N\(_2\)O\(_2\)S\(_2^+\) 233.0413; found: 233.0416.

2-(5-Ethylthio-1,2,4-thiadiazol-3(2H)-ylidene)acetamide (5e) Synthesized according to the general procedure from malonamamidine hydrochloride (138 mg) and bromoethane (131 mg). Eluent: 100:0 to 20:80 hexane:ethyl acetate. White crystalline solid (117 mg, 57% yield). mp 140–142 °C. \( ^1\text{H NMR (400 MHz, CDCl}_3 \) \( \delta \) 6.5 (broad s, 2H), 6.10 (broad s, 2H), 4.36 (q, \( J = 7.1 \) Hz, 2H), 1.43 (t, \( J = 7.4 \) Hz, 3H). \( ^{13}\text{C NMR (101 MHz, CDCl}_3 \) \( \delta \) 166.3, 164.8, 162.4, 114.8, 31.6, 14.4. HRMS (+ESI-QTOF) \( m/z: [M+H]^+ \) calcd for C\(_6\)H\(_{10}\)N\(_3\)OS\(_2^+\) 204.0260; found: 204.0262.

5-Ethylthio-3-methoxy-1,2,4-thiadiazole (5f) Synthesized according to the general procedure from O-methylisourea hemisulfate (121 mg) and bromoethane (131 mg). Solvent: 9:1
acetonitrile:DMPU. Eluent: 100:0 to 95:5 hexane:ethyl acetate. White crystals (83 mg, 47% yield). mp 35–37 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.26, 4.06, 3.26, 3.24, 3.22, 3.20, 1.48, 1.46, 1.45. $^{13}$C NMR (101 MHz, CDCl$_3$) δ 188.8, 171.2, 57.3, 28.4, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{5}$H$_{9}$N$_{2}$O$_{2}$S$_{2}$+ 177.0151; found: 177.0148.

3,5-Bis(ethylthio)-1,2,4-thiadiazole (5h) Synthesized according to the general procedure from S-ethylisothiourea hydrobromide (185 mg) and bromoethane (131 mg). Eluent: 100:0 to 85:15 hexane:ethyl acetate to yield a pale yellow oil (116 mg, 56% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 3.25 (q, $J$ = 7.4 Hz, 2H), 3.2 (q, $J$ = 7.4 Hz, 2H), 1.47 (t, $J$ = 7.4 Hz, 3H), 1.43 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 187.9, 171.1, 28.7, 26.6, 14.8, 14.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{6}$H$_{10}$N$_{2}$S$_{3}$+ 206.0006; found: 206.0003.

5-Ethylthio-3-dimethylamino-1,2,4-thiadiazole (5i) Synthesized according to the general procedure from N,N-dimethylguanidine hemisulfate (136 mg) and bromoethane (131 mg). Solvent: 9:1 acetonitrile:DMPU. Eluent: 100:0 to 80:20 hexane:ethyl acetate. White solid (85 mg, 45% yield). mp 116–118 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 3.165 (q, $J$ = 7.4 Hz, 2H), 3.162 (s, 6H), 1.47 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 186.1, 171.3, 39.1, 28.3, 14.5. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{6}$H$_{12}$N$_{3}$S$_{2}$+ 190.0467; found: 190.0468.

3-(4-Benzylpiperazin-1-yl)-5-ethylthio-1,2,4-thiadiazole (5j) Synthesized according to the general procedure from 4-benzylpiperazine-1-carboximidamide hydroiodide (346 mg) and bromoethane (131 mg). Eluent: 100:0 to 80:20 hexane:ethyl acetate. Colorless liquid (188 mg, 59%). $^1$H NMR (600 MHz, CDCl$_3$) δ 7.37 – 7.30 (m, 4H), 7.30 – 7.24 (m, 1H), 7.30 – 7.24 (m, 1H), 3.72 – 3.67 (m, 4H), 3.55 (s, 2H), 3.16 (q, $J$ = 7.4 Hz, 2H), 2.55 – 2.50 (m, 4H), 1.46 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 186.5, 170.7, 138.1, 129.3, 128.4, 127.3, 63.3, 52.8, 46.8, 28.3, 14.4. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{15}$H$_{21}$N$_{4}$S$_{2}$+ 321.1202; found: 321.1199.
$N^\alpha$-Benzoyl-O-ethyl-$N^\delta$-(5-ethylthio-1,2,4-thiadiazol-3-yl)ornithine (5k) Synthesized according to the general procedure from $N^\alpha$-benzoyl-l-arginine ethyl ester hydrochloride (343 mg) and bromoethane (131 mg). Extracted with ethyl acetate. Eluent: 100:0 to 40:60 hexane:ethyl acetate. White botryoidal crystals (216 mg, 53% yield). mp 92–94 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 – 7.78 (m, 2H), 7.55 – 7.47 (m, 1H), 7.47 – 7.41 (m, 2H), 6.86 (d, $J = 7.7$ Hz, 1H), 5.19 (t, $J = 6.0$ Hz, 1H), 4.85 (td, $J = 7.4$, 5.2 Hz, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.51 – 3.39 (m, 2H), 3.14 (q, $J = 7.4$ Hz, 2H), 2.07 (ddt, $J = 13.4$, 9.6, 5.6 Hz, 1H), 1.89 (dddd, $J = 13.5$, 8.8, 7.2, 6.1 Hz, 1H), 1.84 – 1.67 (m, 2H), 1.44 (t, $J = 7.4$ Hz, 3H), 1.29 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 186.6, 172.6, 169.3, 167.3, 134.1, 131.9, 128.7, 127.2, 61.9, 52.5, 43.1, 30.1, 28.3, 25.8, 14.4, 14.3. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{18}$H$_{24}$N$_4$O$_3$S$_2^+$ 409.1363; found: 409.1365.

2-(4-Chlorophenyl)-4-carbomethoxy-5-((carbomethoxymethyl)thio)imidazole (6) 4-Chlorobenzamidine hydrochloride (2 mmol) and DBU were dissolved in acetonitrile (10 mL), and carbon disulfide (2.2 mmol, 1.1 eq) was added in one portion. The dark orange solution was stirred at room temperature for thirty minutes, and methyl bromoacetate (4.4 mmol, 2.2 eq) was added in one portion, and the solution turned bright yellow, then deep red. The reaction was stirred for 12 hours; then NCS (1.2 eq) was added in one portion, followed by stirring for 30 minutes. The reaction was quenched by the addition of 1M sodium thiosulfate (5 mL) and stirring for 30 minutes, and the mixture was diluted with water (50 mL) and the product was extracted with ethyl acetate (30 mL). The organic layer was washed with water, then 1 M sodium hydroxide, then 1 M hydrochloric acid, and then brine (50 mL each), and then dried over anhydrous magnesium sulfate. The solvent was removed under vacuum, and the residue was subjected to flash chromatography using 100:0 to 75:25 hexane:ethyl acetate. White, fibrous
crystals. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.78 – 7.69 (m, 2H), 7.42 – 7.36 (m, 2H), 4.08 (s, 2H), 3.80 (s, 2H), 3.76 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 168.6, 167.4, 161.4, 158.5, 135.6, 131.9, 131.5, 128.1, 122.0, 53.1, 52.6, 35.2. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{14}$H$_{14}$N$_2$O$_4$S$^+$ 341.0357; found: 341.0355.

5-(2-(Carbmethoxy)ethylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9a) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and methyl acrylate (125 µL, 1.5 eq) instead of an alkyl halide. Extracted with ethyl acetate. Eluent: 100:0 to 85:15 hexane:ethyl acetate; additional recrystallization from hot hexane/ethyl acetate yielded white, fibrous crystals (192 mg, 61% yield). mp 89–90 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.22 – 8.14 (m, 2H), 7.46 – 7.38 (m, 2H), 3.73 (s, 3H), 3.60 (t, $J = 7.0$ Hz, 2H), 2.94 (t, $J = 7.0$ Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 188.1, 171.0, 136.8, 130.8, 129.6, 129.2, 72.4, 41.4, 29.6, 10.1. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{12}$H$_{12}$ClN$_2$O$_2$S$^+$ 315.0023; found: 315.0023.

5-((3-Oxobut-1-yl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9b) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and methyl vinyl ketone (135 µL, 1.5 eq) instead of an alkyl halide. Extracted with ethyl acetate. Eluent: 100:0 to 85:15 hexane:ethyl acetate. Pale yellow oil (32 mg, 11% yield). $^1$H NMR (600 MHz, CDCl$_3$) δ 8.22 – 8.14 (m, 2H), 7.46 – 7.38 (m, 2H), 3.54 (t, $J = 6.7$ Hz, 1H), 3.07 (t, $J = 6.7$ Hz, 1H), 2.21 (s, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 187.5, 171.0, 136.7, 130.8, 129.7, 129.1, 42.9, 30.2, 27.9. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{12}$H$_{12}$ClN$_2$OS$^+$ 299.0074; found: 299.0071.

5-(2-Hydroxy-1-butylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9c) Synthesized according to the general procedure from 4-chlorobenzamidine hydrochloride (191 mg) and 1,2-
epoxybutane (130 µL, 1.5 eq) instead of an alkyl halide. Extracted with ethyl acetate. Eluent: 100:0 to 80:20 hexane:ethyl acetate to yield a colorless oil which solidified upon standing overnight into white, fibrous crystals (143 mg, 48% yield). mp 77–79 ºC. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.19 – 8.11 (m, 2H), 7.48 – 7.39 (m, 2H), 4.05 – 3.93 (m, 1H), 3.58 (dd, \(J = 14.0, 3.2\) Hz, 1H), 3.33 (dd, \(J = 14.0, 7.5\) Hz, 1H), 3.20 (d, \(J = 4.3\) Hz, 1H), 1.69 (qd, \(J = 7.4, 6.3\) Hz, 2H), 1.05 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 188.0, 170.8, 136.7, 130.6, 129.5, 129.0, 72.2, 41.3, 29.5, 10.0. HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for C\(_{12}\)H\(_{15}\)ClN\(_2\)O\(_5\)S\(^2+\) 301.0231; found: 301.0235.

**General procedure for the synthesis of 5-methoxy-1,2,4-thiadiazoles (GP2)**

In a modification of GP1, amidine hydrochloride (1.0 mmol) and DBU (312 mg, 2.05 mmol) were dissolved in methanol (10 mL), followed by the addition of carbon disulfide (114 mg, 1.5 mmol). The pale yellow solution was stirred for 2 hours at 22 ºC, and then ethyl bromide (164 mg, 1.5 mmol) was added in one portion at 22 ºC and the reaction was stirred for 18 hours. The yellow solution was then cooled to 0 ºC, and NCS (147 mg, 1.1 eq). The reaction was stirred at 22 ºC for 30 minutes, and excess NCS was quenched by the addition of 1 M sodium thiosulfate solution (2 mL). The reaction was worked up as in GP1. Thiadiazoles 10 were purified by flash chromatography using 100:0 to 80:20 hexane:ethyl acetate as eluent.

**3-Phenyl-5-methoxy-1,2,4-thiadiazole (10a)** Synthesized according to GP2 from benzamidine hydrochloride (157 mg). Eluent: 100:0 to 75:25 hexane:ethyl acetate. Pale yellow liquid (49.7 mg, 26%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.24 – 8.13 (m, 2H), 7.50 – 7.40 (m, 3H), 4.26 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 191.7, 168.5, 133.1, 130.4, 128.7, 128.0, 60.3. HRMS (+ESI-QTOF) \(m/z\): [M+H]\(^+\) calcd for C\(_9\)H\(_9\)N\(_2\)OS\(^+\) 193.0430; found: 193.0426.

**3-(4-Fluorophenyl)-5-methoxy-1,2,4-thiadiazole (10b)** Synthesized according to GP2 from 4-fluorobenzamidine hydrochloride (175 mg). Eluent: 100:0 to 75:25 hexane:ethyl acetate.
Remaining 11b was removed by adding hexane (1 mL) and cooling to 0°C, followed by filtering through a glass pipet with a cotton plug; the hexane was then removed from the filtrate under vacuum to yield 10b as a white solid (62.9 mg, 30%), containing approximately 5% 11b. mp 73–75 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.24 – 8.15 (m, 2H), 7.16 – 7.08 (m, 3H), 4.24 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 191.7, 167.4, 164.2 (d, $J = 250.2$ Hz), 130.0 (d, $J = 8.7$ Hz), 129.5 (d, $J = 3.1$ Hz), 115.7 (d, $J = 21.9$ Hz), 60.4. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -110.35 (tt, $J = 8.9$, 5.3 Hz). HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{9}$H$_{8}$FN$_{2}$OS$^+$ 211.0336; found: 211.0333.

3-(4-Chlorophenyl)-5-methoxy-1,2,4-thiadiazole (10c) Synthesized according to GP2 from 4-chlorobenzamidine hydrochloride (192 mg). Eluent: 100:0 to 75:25 hexane:ethyl acetate. Remaining 11c was removed as in 10b to yield 10c as a white crystalline solid (95.1 mg, 42%). mp 137–139 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.16 – 8.11 (m, 2H), 7.44 – 7.38 (m, 2H), 4.24 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 191.8, 167.4, 136.4, 131.6, 129.3, 128.9, 60.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{9}$H$_{8}$ClN$_{2}$OS$^+$ 227.0040; found: 227.0038.

5-Methoxy-3-(3-methoxyphenyl)-1,2,4-thiadiazole (10d) Synthesized according to GP2 from 3-methoxybenzamidine hydrochloride (187 mg). Eluent: 100:0 to 80:20 hexane:ethyl acetate. Remaining 11d was removed as in 10b. White crystalline solid (108 mg, 49%). mp 73–75 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.81 (dt, $J = 7.6$, 1.3 Hz, 1H), 7.75 (dd, $J = 2.7$, 1.5 Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 6.99 (ddd, $J = 8.2$, 2.7, 1.0 Hz, 1H), 4.24 (s, 3H), 3.88 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 191.5, 168.2, 159.8, 134.3, 129.6, 120.5, 116.7, 112.5, 60.2, 55.4. HRMS (+ESI-QTOF) m/z: [M+H]$^+$ calcd for C$_{10}$H$_{11}$N$_{2}$O$_{2}$S$^+$ 223.0536; found: 223.0536.

5-Methoxy-3-(4-nitrophenyl)-1,2,4-thiadiazole (10e) Synthesized according to GP2 from 4-nitrobenzamidine hydrochloride (202 mg). Eluent: 100:0 to 75:25 hexane:ethyl acetate.
Pale yellow crystalline solid (151 mg, 64%). mp 138–140 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.40 – 8.34 (m, 1H), 8.31 – 8.26 (m, 1H), 4.28 (s, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 192.2, 166.1, 148.8, 138.4, 128.8, 124.0, 60.6. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_9$H$_8$N$_2$O$_3$S$^+$ 238.0281; found: 238.0282.

**General procedure for the synthesis of 2-methoxy-4,6-disubstituted-1,3,5-triazines (GP3)**

In a modification of GP1, amidine hydrochloride (2.0 mmol) and DBU (624 mg, 4.1 mmol) were dissolved in methanol (15 mL), followed by the addition of carbon disulfide (76 mg, 1 mmol) and ethyl bromide (273 mg, 2.5 mmol) in one portion at 22 °C; the reaction was stirred at room temperature for 2 hours, and then heated at reflux 6 hours. The excess methanol was removed from the pale yellow solution under vacuum, and the reaction was worked up as in GP1. Triazines were purified by flash chromatography using 100:0 to 80:20 hexane:ethyl acetate as eluent.

**2-Methoxy-4,6-diphenyl-1,3,5-triazine (11a)** Synthesized according to GP3 from benzamidine hydrochloride (313 mg). White crystalline solid (87.0 mg, 33%). mp 109–111 °C. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.69 – 8.62 (m, 4H), 7.62 – 7.57 (m, 2H), 7.56 – 7.51 (m, 4H), 4.23 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 173.7, 172.0, 135.8, 132.8, 129.2, 128.7, 55.1. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{16}$H$_{14}$N$_3$O$^+$ 264.1131; found: 264.1129.

**4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine (11b)** Synthesized according to GP3 from 4-fluorobenzamidine hydrochloride (349 mg). White crystalline solid (136 mg, 45%). mp 173–174 °C. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.66 – 8.59 (m, 4H), 7.23 – 7.16 (m, 4H), 4.20 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 172.6, 171.8, 165.9 (d, $J$ = 253.4 Hz), 131.7 (d, $J$ = 2.9 Hz), 131.4 (d, $J$ = 8.9 Hz), 115.7 (d, $J$ = 21.8 Hz), 55.0. $^{19}$F{$^1$H} NMR (565 MHz, CDCl$_3$) δ -106.6. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{16}$H$_{12}$F$_2$N$_3$O$^+$ 300.0943; found: 300.0941.
4,6-Bis(4-chlorophenyl)-2-methoxy-1,3,5-triazine (11c) Synthesized according to GP3 from 4-chlorobenzamidine hydrochloride (382 mg). White fibrous solid (130 mg, 39%). mp 192–194 ºC. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.58 – 8.53 (m, 4H), 7.52 – 7.47 (m, 4H), 4.21 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 172.9, 172.0, 139.3, 134.1, 130.5, 129.1, 55.3. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{16}$H$_{12}$Cl$_2$N$_3$O + 332.0352; found: 332.0353.

4,6-Bis(3-methoxyphenyl)-2-methoxy-1,3,5-triazine (11d) Synthesized according to GP3 from 3-methoxybenzamidine hydrochloride (373 mg). White fibrous solid (109 mg, 34%). mp 126–128 ºC. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.25 (dt, $J$ = 7.7, 1.3 Hz, 2H), 8.17 (dd, $J$ = 2.7, 1.5 Hz, 2H), 7.44 (t, $J$ = 7.9 Hz, 2H), 7.14 (ddd, $J$ = 8.2, 2.7, 1.0 Hz, 2H), 4.22 (s, 3H), 3.93 (s, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 173.4, 171.8, 159.9, 137.1, 129.6, 121.6, 118.9, 113.7, 55.5, 55.1. HRMS (+ESI-QTOF) $m/z$: [M+H]$^+$ calcd for C$_{18}$H$_{18}$N$_3$O$_3$+ 324.1343; found: 324.1342.

4,6-Bis(4-chlorophenyl)-2-ethylthio-1,3,5-triazine (14) In acetonitrile (10 mL) was dissolved DBU (312 mg, 2.05 mmol) and 4-chlorobenzamidine hydrochloride (192 mg). Carbon disulfide (38 mg, 0.5 mmol) and ethyl bromide (273 mg, 2.5 eq) were added together, and the reaction was stirred at 22 ºC for two hours, and then at reflux for six hours. The yellow solution was poured into water (50 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with water, then 1 M sodium hydroxide, then brine (10 mL each). The solution as dried over anhydrous magnesium sulfate, and the solvent was removed under vacuum. The crude product was purified by column chromatography using and eluent gradient of 100:0 to 75:25 hexane:ethyl acetate to yield a quantity of 3b (38.9 mg, 30% based on CS$_2$) as bright yellow-orange needles, and the title compound as a white fibrous solid (33.4 mg, 18%). mp 173–174 ºC. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.59 – 8.49 (m, 4H), 7.57 – 7.45 (m, 4H), 3.31 (q, $J$ = 7.3 Hz, 2H), 1.51 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 183.3, 169.4, 139.2,
134.2, 130.4, 129.1, 25.2, 14.5. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C_{17}H_{14}Cl_{2}N_{3}S^{+}
362.0280; found: 362.0279.

3-Phenyl-5-ethoxy-1,2,4-thiadiazole (15) Synthesized according to GP2 from benzamidine hydrochloride (157 mg), with the modification of using 1:1 ethanol:acetonitrile instead of methanol as the solvent. Eluent: 100:0 to 80:20 hexane:ethyl acetate. Pale yellow liquid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.24 – 8.17 (m, 2H), 7.51 – 7.39 (m, 3H), 4.61 (q, \(J = 7.1\) Hz, 2H), 1.52 (t, \(J = 7.1\) Hz, 3H). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 191.0, 168.5, 133.2, 130.3, 128.6, 127.9, 70.1, 14.6. HRMS (+ESI-QTOF) m/z: [M+H]+ calcd for C_{10}H_{11}N_{2}OS^{+} 207.0587; found: 207.0588.

References


Appendix

Ethyl 1-imidazolecarbodithioate (2) $^1$H NMR (400 MHz, CDCl$_3$)

Ethyl 1-imidazolecarbodithioate (2) $^{13}$C NMR (101 MHz, CDCl$_3$)
S-Ethyl N-(α-iminobenzyl)dithiocarbamate (3a) $^1$H NMR (400 MHz, CDCl$_3$)

\[
\text{S-ethyl N-(α-iminobenzyl)dithiocarbamate (3a) } ^1\text{H NMR (400 MHz, CDCl}_3\text{)}
\]

\[
\text{S-ethyl N-(α-iminobenzyl)dithiocarbamate (3a) } ^1\text{H NMR (400 MHz, DMSO-}d_6\text{)}
\]

\[
\text{S-ethyl N-(α-iminobenzyl)dithiocarbamate (3a) } ^1\text{H NMR (400 MHz, DMSO-}d_6\text{)}
\]
$S$-Ethyl $N$-(α-iminobenzyl)dithiocarbamate (3a) $^{13}$C NMR (101 MHz, CDCl$_3$)

$S$-Ethyl $N$-(α-iminobenzyl)dithiocarbamate (3a) $^{13}$C NMR (101 MHz, DMSO-$d_6$)
**S-Ethyl N-(α-imino-4-chlorobenzyl)dithiocarbamate (3b)**

$^1$H NMR (600 MHz, CDCl$_3$)

![NMR Spectroscopy](image)

**S-Ethyl N-(α-imino-4-chlorobenzyl)dithiocarbamate (3b)**

$^1$H NMR (600 MHz, CDCl$_3$)

![NMR Spectroscopy](image)
5-Ethylthio-3-phenyl-1,2,4-thiadiazole (4a) \({}^1\text{H}\) NMR (400 MHz, CDCl$_3$)

\[
\text{H NMR (400 MHz, CDCl}_3\text{)}
\]

5-Ethylthio-3-phenyl-1,2,4-thiadiazole (4a) \(^{13}\text{C}\) NMR (101 MHz, CDCl$_3$)

\[
\text{C NMR (101 MHz, CDCl}_3\text{)}
\]
5-Methylthio-3-phenyl-1,2,4-thiadiazole (4b) $^1$H NMR (400 MHz, CDCl$_3$)

5-Methylthio-3-phenyl-1,2,4-thiadiazole (4b) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-Phenyl-5-propylthio-1,2,4-thiadiazole (4c) $^1$H NMR (400 MHz, CDCl$_3$)

3-Phenyl-5-propylthio-1,2,4-thiadiazole (4c) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Isopropylthio-3-phenyl-1,2,4-thiadiazole (4d) $^1$H NMR (400 MHz, CDCl$_3$)

5-Isopropylthio-3-phenyl-1,2,4-thiadiazole (4d) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Isobutylthio-3-phenyl-1,2,4-thiadiazole (4e) $^1$H NMR (400 MHz, CDCl$_3$)

5-Isobutylthio-3-phenyl-1,2,4-thiadiazole (4e) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-(Cyclopropylmethylthio)-3-phenyl-1,2,4-thiadiazole (4f) $^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum](image1)

5-(Cyclopropylmethylthio)-3-phenyl-1,2,4-thiadiazole (4f) $^{13}$C NMR (101 MHz, CDCl$_3$)

![NMR spectrum](image2)
5-Allylthio-3-phenyl-1,2,4-thiadiazole (4g) $^1$H NMR (400 MHz, CDCl$_3$)

5-Allylthio-3-phenyl-1,2,4-thiadiazole (4g) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Benzylthio-3-phenyl-1,2,4-thiadiazole (4h) ¹H NMR (400 MHz, CDCl₃)

5-Benzylthio-3-phenyl-1,2,4-thiadiazole (4h) ¹³C NMR (101 MHz, CDCl₃)
5-Citronellythio-3-phenyl-1,2,4-thiadiazole (4i) \(^1\)H NMR (600 MHz, CDCl\(_3\))

5-Citronellythio-3-phenyl-1,2,4-thiadiazole (4i) \(^{13}\)C NMR (151 MHz, CDCl\(_3\))
5-Ethylthio-3-(4-fluorophenyl)-1,2,4-thiadiazole (4j) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-(4-fluorophenyl)-1,2,4-thiadiazole (4j) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Ethylthio-3-(4-fluorophenyl)-1,2,4-thiadiazole (4j) \(^{19}\text{F NMR (376 MHz, CDCl}_3\)}

\[
\begin{align*}
\text{F} & \quad \text{N} \quad \text{S} \\
\text{N} & \quad \text{S} \\
\end{align*}
\]

3-(4-Chlorophenyl)-5-ethylthio-1,2,4-thiadiazole (4k) \(^{1}\text{H NMR (400 MHz, CDCl}_3\)}

\[
\begin{align*}
\text{Cl} & \quad \text{N} \quad \text{S} \\
\text{N} & \quad \text{S} \\
\end{align*}
\]
3-(4-Chlorophenyl)-5-ethylthio-1,2,4-thiadiazole (4k) $^{13}$C NMR (101 MHz, CDCl$_3$)

3-(4-Chlorophenyl)-5-methylthio-1,2,4-thiadiazole (4l) $^1$H NMR (400 MHz, CDCl$_3$)
3-(4-Chlorophenyl)-5-methylthio-1,2,4-thiadiazole (4l) $^{13}$C NMR (101 MHz, CDCl$_3$)

$^{13}$C NMR spectra showing chemical shifts for various carbon atoms.

3-(4-Chlorophenyl)-5-methylthio-1,2,4-thiadiazole (4m) $^1$H NMR (600 MHz, CDCl$_3$)

$^1$H NMR spectra showing chemical shifts for various hydrogen atoms.
3-(4-Chlorophenyl)-5-methylthio-1,2,4-thiadiazole (4m) $^{13}$C NMR (151 MHz, CDCl$_3$)

5-((2-bromo-5-methoxybenzyl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (4n) $^1$H NMR (600 MHz, CDCl$_3$)
5-((2-bromo-5-methoxybenzyl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (4n) $^{13}$C NMR (151 MHz, CDCl$_3$)

3-(4-Bromophenyl)-5-ethylthio-1,2,4-thiadiazole (4o) $^1$H NMR (400 MHz, CDCl$_3$)
3-(4-Bromophenyl)-5-ethylthio-1,2,4-thiadiazole (4o) $^{13}$C NMR (101 MHz, CDCl$_3$)

5-Ethylthio-3-(4-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (4p) $^1$H NMR (400 MHz, CDCl$_3$)
5-Ethylthio-3-(4-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (4p) $^{13}$C NMR (101 MHz, CDCl$_3$)

5-Ethylthio-3-(4-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (4p) $^{19}$F NMR (376 MHz, CDCl$_3$)
5-Ethylthio-3-(4-methoxyphenyl)-1,2,4-thiadiazole (4q) \(^1\)H NMR (400 MHz, CDCl\(_3\))

\[ \text{NMR spectrum image} \]

5-Ethylthio-3-(4-methoxyphenyl)-1,2,4-thiadiazole (4q) \(^{13}\)C NMR (101 MHz, CDCl\(_3\))

\[ \text{NMR spectrum image} \]
5-Ethylthio-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4r) \(^1\)H NMR (400 MHz, CDCl\(_3\))

\[
\begin{align*}
\text{Thia-T21.1.fid} & \quad \text{NMR spectrum}
\end{align*}
\]

5-Ethylthio-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4r) \(^1\)C NMR (101 MHz, CDCl\(_3\))

\[
\begin{align*}
\text{Thia-T21.2.fid} & \quad \text{NMR spectrum}
\end{align*}
\]
5-((2-Methoxyethyl)thio)-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4s) $^1$H NMR (600 MHz, CDCl$_3$)

5-((2-Methoxyethyl)thio)-3-(3-methoxyphenyl)-1,2,4-thiadiazole (4s) $^{13}$C NMR (151 MHz, CDCl$_3$)
**tert-Butyl (2-((3-phenyl-1,2,4-thiadiazol-5-yl)thio)ethyl)carbamate (4t)**

**1H NMR (600 MHz, CDCl₃)**

**13C NMR (151 MHz, CDCl₃)**
5-Ethylthio-3-(3-toluyl)-1,2,4-thiadiazole (4u) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-(3-toluyl)-1,2,4-thiadiazole (4u) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Ethylthio-3-(4-nitrophenyl)-1,2,4-thiadiazole (4v) \(^1\)H NMR (400 MHz, CDCl\(_3\))

5-Ethylthio-3-(4-nitrophenyl)-1,2,4-thiadiazole (4v) \(^1\)C NMR (101 MHz, CDCl\(_3\))
**5-Ethylthio-3-(pyridin-2-yl)-1,2,4-thiadiazole (4w) **

$^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum of 5-Ethylthio-3-(pyridin-2-yl)-1,2,4-thiadiazole (4w)](image)

$^{13}$C NMR (101 MHz, CDCl$_3$)

![C NMR spectrum of 5-Ethylthio-3-(pyridin-2-yl)-1,2,4-thiadiazole (4w)](image)
5-Ethylthio-3-(pyridin-4-yl)-1,2,4-thiadiazole (4x) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-(pyridin-4-yl)-1,2,4-thiadiazole (4x) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Ethylthio-3-(pyrazin-2-yl)-1,2,4-thiadiazole (4y) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-(pyrazin-2-yl)-1,2,4-thiadiazole (4y) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Ethylthio-3-methyl-1,2,4-thiadiazole (5a) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-methyl-1,2,4-thiadiazole (5a) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-Cyclopropyl-5-ethythio-1,2,4-thiadiazole (5b) $^1$H NMR (400 MHz, CDCl$_3$)

3-Cyclopropyl-5-ethythio-1,2,4-thiadiazole (5b) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-(2,6-Dichlorobenzyl)-5-ethylthio-1,2,4-thiadiazole (5c) $^1$H NMR (400 MHz, CDCl$_3$)

3-(2,6-Dichlorobenzyl)-5-ethylthio-1,2,4-thiadiazole (5c) $^{13}$C NMR (101 MHz, CDCl$_3$)
Ethyl 2-(5-ethylthio-1,2,4-thiadiazol-3-yl)acetate (5d) $^1$H NMR (400 MHz, CDCl$_3$)

**Chemical Structure**

![Chemical Structure](image)

**NMR Spectra**

**$^1$H NMR Spectra**

- Chemical shifts: 0.00 - 5.0 ppm
- Peaks at 1.38, 1.40, 1.42, 1.44, 1.46 ppm
- Peaks at 2.96, 2.98, 3.00 ppm
- Peaks at 4.33, 4.35, 4.37, 4.39 ppm
- Peaks at 5.76 ppm

**$^{13}$C NMR Spectra**

- Chemical shifts: 10.0 - 150.0 ppm
- Peaks at 13.5, 14.5 ppm
- Peaks at 28.1, 77.1 ppm
- Peaks at 109.4 ppm
- Peaks at 163.2, 165.3 ppm

SN

N

S

O

H

SN

N

S

O

H
2-(5-(Ethylthio)-1,2,4-thiadiazol-3-yl)acetamide (5e) $^1$H NMR (400 MHz, CDCl$_3$)

$$\text{H}_2\text{N} \quad \begin{array}{c} \text{O} \\ \end{array} \quad \text{N} \quad \begin{array}{c} \text{S} \\ \end{array} \quad \text{S}$$

1H NMR (400 MHz, CDCl$_3$)

2-(5-(Ethylthio)-1,2,4-thiadiazol-3-yl)acetamide (5e) $^{13}$C NMR (101 MHz, CDCl$_3$)

$$\text{H}_2\text{N} \quad \begin{array}{c} \text{O} \\ \end{array} \quad \text{N} \quad \begin{array}{c} \text{S} \\ \end{array} \quad \text{S}$$
5-Ethylthio-3-methoxy-1,2,4-thiadiazole (5f) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-methoxy-1,2,4-thiadiazole (5f) $^{13}$C NMR (101 MHz, CDCl$_3$)
3,5-Bis(ethylthio)-1,2,4-thiadiazole (5h) $^1$H NMR (400 MHz, CDCl$_3$)

3,5-Bis(ethylthio)-1,2,4-thiadiazole (5h) $^{13}$C NMR (101 MHz, CDCl$_3$)
5-Ethylthio-3-dimethylamino-1,2,4-thiadiazole (5i) $^1$H NMR (400 MHz, CDCl$_3$)

5-Ethylthio-3-dimethylamino-1,2,4-thiadiazole (5i) $^{13}$C NMR (101 MHz, CDCl$_3$)
3-(4-Benzylpiperazin-1-yl)-5-ethylthio-1,2,4-thiadiazole (5j) $^1$H NMR (600 MHz, CDCl$_3$)

1H NMR (600 MHz, CDCl$_3$)

3-(4-Benzylpiperazin-1-yl)-5-ethylthio-1,2,4-thiadiazole (5j) $^{13}$C NMR (151 MHz, CDCl$_3$)

13C NMR (151 MHz, CDCl$_3$)
$N^\alpha$-Benzoyl-$O$-ethyl-$N^\alpha$(5-ethylthio-1,2,4-thiadiazol-3-yl)ornithine (5k) $^1$H NMR (400 MHz, CDCl$_3$)
$N^\alpha$-Benzoyl-$O$-ethyl-$N^\alpha$-(5-ethylthio-1,2,4-thiadiazol-3-yl)ornithine (5k) HMQC (400 MHz, CDCl₃)

2-(4-Chlorophenyl)-4-carbmethoxy-5-((carbmethoxymethyl)thio)imidazole (6) $^1$H NMR (400 MHz, CDCl₃)
2-(4-Chlorophenyl)-4-carbomethoxy-5-((carbomethoxymethyl)thio)imidazole (6) $^{13}$C NMR (101 MHz, CDCl$_3$)

5-(2-(Carbomethoxy)ethylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9a) $^1$H NMR (400 MHz, CDCl$_3$)
5-(2-(Carbomethoxy)ethylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9a) \(^{13}\text{C}\) NMR (101 MHz, CDCl\(_3\))

5-((3-Oxobut-1-yl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9b) \(^1\text{H}\) NMR (600 MHz, CDCl\(_3\))
5-((3-Oxobut-1-yl)thio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9b) $^{13}$C NMR (151 MHz, CDCl$_3$)

5-(2-Hydroxy-1-butylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9c) $^1$H NMR (400 MHz, CDCl$_3$)
5-(2-Hydroxy-1-butylthio)-3-(4-chlorophenyl)-1,2,4-thiadiazole (9c) $^{13}$C NMR (101 MHz, CDCl$_3$)

![NMR spectrum of 9c](image)

3-Phenyl-5-methoxy-1,2,4-thiadiazole (10a) $^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum of 10a](image)
3-Phenyl-5-methoxy-1,2,4-thiadiazole (5a) $^{13}$C NMR (101 MHz, CDCl$_3$)

3-(4-Fluorophenyl)-5-methoxy-1,2,4-thiadiazole (10b) $^1$H NMR (400 MHz, CDCl$_3$)
3-(4-Fluorophenyl)-5-methoxy-1,2,4-thiadiazole (10b) $^{13}$C NMR (101 MHz, CDCl$_3$)

![C NMR Spectrum](image)

3-(4-Fluorophenyl)-5-methoxy-1,2,4-thiadiazole (10b) $^{19}$F NMR (379 MHz, CDCl$_3$)

![F NMR Spectrum](image)
3-(4-Chlorophenyl)-5-methoxy-1,2,4-thiadiazole (10c) $^1$H NMR (600 MHz, CDCl$_3$)

\[
\text{\begin{tabular}{|c|}
\hline
3.05 \text{ ppm} \\
\hline
\end{tabular}}
\]

3-(4-Chlorophenyl)-5-methoxy-1,2,4-thiadiazole (10c) $^{13}$C NMR (151 MHz, CDCl$_3$)

\[
\text{\begin{tabular}{|c|}
\hline
60.38 \text{ ppm} \\
\hline
\end{tabular}}
\]
5-Methoxy-3-(3-methoxyphenyl)-1,2,4-thiadiazole (10d) $^1$H NMR (600 MHz, CDCl$_3$)

5-Methoxy-3-(3-methoxyphenyl)-1,2,4-thiadiazole (10d) $^{13}$C NMR (151 MHz, CDCl$_3$)
5-Methoxy-3-(4-nitrophenyl)-1,2,4-thiadiazole (10e) $^1$H NMR (600 MHz, CDCl$_3$)

5-Methoxy-3-(4-nitrophenyl)-1,2,4-thiadiazole (10e) $^{13}$C NMR (151 MHz, CDCl$_3$)
2-Methoxy-4,6-diphenyl-1,3,5-triazine (11a) $^1$H NMR (400 MHz, CDCl$_3$)

2-Methoxy-4,6-diphenyl-1,3,5-triazine (11a) $^{13}$C NMR (101 MHz, CDCl$_3$)
4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine (11b) \(^1\)H NMR (600 MHz, CDCl\(_3\))

![NMR spectrum of 4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine](image1)

4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine (11b) \(^1\)C NMR (151 MHz, CDCl\(_3\))

![NMR spectrum of 4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine](image2)
4,6-Bis(4-fluorophenyl)-2-methoxy-1,3,5-triazine (11b) $^{19}$F $^1$H NMR (565 MHz, CDCl$_3$)

4,6-Bis(4-chlorophenyl)-2-methoxy-1,3,5-triazine (11c) $^1$H NMR (400 MHz, CDCl$_3$)
4,6-Bis(4-chlorophenyl)-2-methoxy-1,3,5-triazine (11c) $^{13}$C NMR (101 MHz, CDCl$_3$)

4,6-Bis(3-methoxyphenyl)-2-methoxy-1,3,5-triazine (11d) $^1$H NMR (400 MHz, CDCl$_3$)
4,6-Bis(3-methoxyphenyl)-2-methoxy-1,3,5-triazine (11d) $^{13}$C NMR (101 MHz, CDCl$_3$)

4,6-Bis(4-chlorophenyl)-2-ethylthio-1,3,5-triazine (14) $^1$H NMR (400 MHz, CDCl$_3$)
4,6-Bis(4-chlorophenyl)-2-ethylthio-1,3,5-triazine (14) $^{13}$C NMR (101 MHz, CDCl$_3$)

3-Phenyl-5-ethoxy-1,2,4-thiadiazole (15) $^1$H NMR (400 MHz, CDCl$_3$)
3-Phenyl-5-ethoxy-1,2,4-thiadiazole (15) $^{13}$C NMR (101 MHz, CDCl$_3$)
CHAPTER 9. GENERAL CONCLUSIONS

Overview

Nature provides an abundance of new compounds to discover, investigate, and derivatize. Evolution favors the biosynthesis of new repellents and pesticides, and pests also evolve a host of defenses against these compounds. Whether the resistance results from physical or behavioral modifications, changes in metabolism, or mutations in the active site of the molecule of interest, the result is the same: the xenobiotic compound no longer has the desired efficacy against the target organism. Resistance is often hastened through poor pest management practices, including the repetitive use of compounds with the same mechanism of action and the application of insufficient quantities of the treatment.\textsuperscript{1-2}

To counter the increased prevalence of resistance, compounds with new mechanisms of action or different chemical structures that resist metabolism are crucial for agriculture, vector control, and human medicine. The process of discovering new compounds using traditional \textit{de novo} screenings from small-molecule libraries, followed by the rational development of lead compounds into drug-like molecules, can be prohibitively expensive.\textsuperscript{3-5} The biorational approach of modifying natural products with known or suspected biological activity can often shorten the development process of these compounds, and this tactic was used in this dissertation to develop new insect repellents by the esterification of monoterpenoids. A similar iterative biorational approach led from cinnamaldehyde to the development of styryl sulfides and (alkylthio)azoles as potential nematicides.

The biorational modification of natural compounds remains a vital route to developing new pesticides and pharmaceutical compounds. Identifying natural compounds with a desired mechanism of action dramatically reduces the effort needed to develop lead compounds for
future modification, especially compared to small molecule screening methods.\textsuperscript{6-7} Evolution has provided organisms the chemical means to protect themselves, and exploiting the biochemistry behind these compounds allows us to harness the power of chemicals to improve the quality of life for humanity through a biorational approach.

**Arthropod Repellents**

Although the prevention of arthropod-vectored diseases has often focused on insecticide use, repellents remain an essential part of the equation in preventing bites and subsequent disease transmission—arguably, the use of repellents has become all the more critical as mosquito resistance to numerous insecticides, including many organophosphates pyrethroids, threatens the progress made against the diseases these vectors carry.\textsuperscript{8-9} Commercial repellents, including DEET, IR3535, and picaridin, are familiar to many consumers as common insect repellents. However, these compounds are contact repellents, and only repel arthropods that come into direct contact with a treated surface. This mode of repellency potentially leaves individuals exposed if, for example, a portion of the skin is left treated and a mosquito or other disease vector comes into contact with this surface. Spatial repellents, by way of contrast, exhibit a repellent action through space by slowly vaporizing off of a treated surface, and deterring insects from coming closer.

Monoterpenoid and phenylpropanoid alcohols have long been exploited as insect repellents, frequently in a mixture in the form of a plant essential oil. While these compounds do indeed offer some spatial repellency immediately after application to the skin, rapid evaporation of the actively repellent compounds results in loss of efficacy. By using a biorational approach to increase the molecular weight of monoterpenoids and phenylpropanoids through esterification, the longevity of these compounds was substantially increased, with some products, such as monocyclic monoterpenoid isovalerates and several citronellyl and citronellate esters, providing
excellent spatial repellency after seven hours of exposure to air. This substantial improvement in
the longevity of these repellents may make these compounds useful for excluding mosquitoes
from enclosed areas.

As a modification of the typical carboxylic acid ester of monoterpenoids and
phenylpropanoids, a series of carbonate esters were also produced. These analogs of the
carboxylate esters contain an additional oxygen atom and are of considerable interest given the
high spatial repellency of some of these compounds, notably the series of ethyl monoterpenoid
carbonates, in a short-term repellency study. The carbonate ester class of repellents is poorly-
explored compared to other series of molecules such as carboxylic esters, alcohols, and amides.
Although these compounds have not yet been studied for their long-term spatial repellency, the
carbonate esters represent a promising new class of spatial repellents.

Nematicides

Plant-parasitic nematodes are of tremendous agricultural concern, causing considerable
economic damage to crops and threatening food security. Since its devastating introduction into
the United States, soybean cyst nematode has been best controlled through the use of SCN-
resistant soybean varieties. The evolution and spread of SCN populations with increased
virulence on these resistant plants, compounded by the lack of diversity in available resistant
strains and the lack of commercially-viable nematicides available to reduce this plant-parasitic
burden, is potentially catastrophic to the soybean industry,\textsuperscript{10-11} and there is a significant need for
new methods of controlling these nematodes.

Although all nematodes share a similar body plan, this group of organisms is incredibly
diverse.\textsuperscript{12} SCN and other plant-parasitic nematodes undeniably are of great concern, but it is not
desirable to destroy all nematode live in the soil, as many nematodes can be beneficial and are
essential parts of the soil fauna.\textsuperscript{13-14} Cinnamaldehyde has some nematicidal activity; however,
this phenylpropanoid is susceptible to autoxidation and microbial degradation, reducing its utility as an antinematicidal compound. β-Nitrostyrenes and styryl ketones provide a more-potent alternative to cinnamaldehyde, as do various substituted (alkylthio)azoles. While the mechanism of action of these azoles remains to be determined, several new compounds in this group, notably an isothiazole and a pyrazole, induced significant esterase activity in *C. elegans*, suggesting potential upregulation of metabolic activity.

Importantly, the estimated bioactivities of these compounds against *C. elegans* and SCN showed low correlation, suggesting that it is possible to selectively target plant-parasitic nematodes, like SCN, while leaving other nematodes relatively unharmed. However, a fluorescence-based assay using *C. elegans* was useful in determining which compounds may be effective against SCN and provides a method of whole-organism high-throughput screening of newly synthesized compounds. Given the thousands of strains of gene-variant *C. elegans* available for metabolic assays, gene expression, and knockout studies, this fluorescence-based bioassay may also prove useful far beyond the scope of nematicide screening.

**The Role of Chemistry**

Exploring the chemical space around natural products often requires the creation of new chemical methods to produce analogs of interest. Although new reactions are usually developed with a specific need in mind, these methods are frequently valuable for broader applications. In this dissertation, two new methods were developed—one for the production of ketene dithioacetals, and another focused on 5-alkylthio-1,2,4-thiadiazoles.

Ketene dithioacetals (KDTAs) are readily produced by reacting an acidic carbon with carbon disulfide and an alkyl halide in the presence of a base; however, methods for making KDTAs without electron-withdrawing groups remain scarce. Instead of relying on electron-withdrawing groups in the starting material, a one-pot reaction was developed wherein the
readily-available dimethyl methylphosphonate was deprotonated with lithium diisopropylamide in the presence of an organic disulfide to produce a bis(alkylthio)-methylphosphonate, which undergoes a Horner-Wadsworth-Emmons reaction with an aldehyde to produce a KDTA. Although the method was developed to produce analogs of nematicidal styryl sulfides, it is likely that this convenient, one-pot synthesis of KDTAs from aldehydes will have broader appeal, given the utility of KDTAs as synthetic intermediates.

The success of styryl sulfides as nematicides also ultimately led to the synthesis of 5-alkylthio-1,2,4-thiadiazoles (ATTDs). Methods of synthesizing alkylthio-substituted pyrazoles, isoxazoles, and isothiazoles were already available in the chemical literature. However, the analogous ATTDs required multiple steps or unattractive starting materials. Amidines, which are readily available from the corresponding nitriles, served as useful building blocks for the synthesis of ATTDs from carbon disulfide and an alkyl halide, with the affordable N-chlorosuccinimide as an oxidizing agent. While the original scope of the reaction was focused on benzamidine derivatives, the reaction also worked with heteroarylamidines, and alkylamidines, as well as guanidines and isoureas. Remarkably, even a protected arginine served as a suitable substrate, signifying that this method may have broad application, particularly since other methods require multiple steps to form the substituted thiadiazole ring.

Outlook

We live in a global society, characterized by the spread of information and technology, much of which is to the benefit of humankind. However, the outlook is not entirely rosy. A growing world population, with growing inequality, helps to highlight issues like food insecurity and unequal disease prevalence. The advancement of science does not occur in a vacuum, and politics and current events can produce significant pressure on the development of new chemical technologies.
Mosquitoes and other arthropods remain a significant threat to human health throughout the world. The Zika virus epidemic of 2015 – 2016 resulted in a significant boost in the interest and funding of mosquito control technologies. Unfortunately, mosquitoes are becoming resistant to many common pesticide classes, including pyrethroids, carbamates, and organophosphates. Of additional concern is the growing resistance of disease-causing organisms, such as malaria, to first-line medications. In many locations, malaria is already resistant to the common antimalarial drugs mefloquine, sulfadoxine-pyrimethamine, and chloroquine. Of additional current concern is the unproven, off-label use of chloroquine and hydroxychloroquine as a treatment or prophylactic for the novel coronavirus disease COVID-19, which could further undermine our current ability to treat malaria. Recently, artemisinin, which has become more affordable and attractive for use against chloroquine-resistant malaria, the severe selection pressure of the widespread use of a single control method has led to the development of malaria resistance to artemisinin despite efforts to mitigate and contain any such resistant populations.

By reducing crop yields, plant-parasitic nematodes represent a significant agricultural problem, while serving as an example of the danger of invasive species. The introduction of SCN to the US and other soybean-producing areas around the world continues to vex farmers. International travel and trade continue to serve as a conduit for the transfer of these pests to new continents, where they continue to wreak havoc. The problem of plant-parasitic nematodes is further exacerbated by the lack of affordable treatments to reduce plant damage.

The development of resistance in a pest or pathogen is of great concern, and new classes of chemicals are often needed to control populations that become resistant to multiple classes of treatments. The development of any new drug or pesticide requires years of effort and enormous
sums of money, and new lead compounds for further exploration are few and far between.

Natural products often provide a shortcut in the discovery process by acting as lead compounds, and the production of biorational analogs will likely remain an essential step in the development of pesticides and pharmaceuticals for the foreseeable future.

References


