A Thesis
Entitled
Importance of the α,β-Unsaturated Ketone in Methuosis-Inducing Compounds
By
Jennifer Offenbacher
Submitted to the Graduate Faculty as partial fulfillment of the requirements for the
Master of Science Degree in
Medicinal Chemistry

Paul W. Erhardt, PhD, Committee Chair

Christopher J. Trabbic, PhD, Committee Member

Jeffery Sarver, PhD, Committee Member

Patricia R. Komuniecki, PhD, Dean
College of Graduate Studies

University of Toledo
December 2015
Methuosis is a novel type of nonapoptotic cell death in which vacuoles form and swell until the cell membrane bursts. Several indole-based chalcones have been found to induce methuosis. These indole-based chalcones contain α,β-unsaturated ketones which may undergo Michael addition. However, the heterocycles on either side of the α,β-unsaturated ketone have a less studied effect on Michael addition. Also, the metabolites of methuosis-inducing chalcones are reduced forms of the α,β-unsaturated ketone. These metabolites are synthesized as a part of this thesis. Synthesis of analogues on the pyridinyl ring, primarily extending the nitrogen out of the ring and modifying the electronics is reported herein.
Acknowledgements

I would first like to thank Dr. Erhardt, my advisor, for his help during my Master’s program. His love and excitement for chemistry is inspiring and encouraging which is a true blessing.

Chris Trabbic, Ph D., one of my committee members, has had a huge influence with my success as a chemist. With all of the training he has provided for me, it has helped me mold into the chemist I am today. He has been there to answer my day-to-day questions and has always been available for problem solving situations.

Jeff Sarver, Ph D., my other committee member, has helped me throughout my analytical work and has trained me in both HPLC and LCMS. Jeff has helped me with kinetic calculations and understanding proper kinetic studies.

I would like to thank the remaining members of the CD3 for day-to-day brainstorming and problem solving. Finally, my oldest brother, Adam, has played a huge role in achieving my dreams by always helping me with my academic and career decisions. Without his help and input I would not be where I am today. My parents and other brother, Nathan, has always given me support and advice. Also, my boyfriend, Mike, who has been with me during my graduate career and has given me support and tolerated the crazy hours and stressful moments.
# Table of Contents

Abstract ........................................................................................................ iii

Acknowledgements ......................................................................................... iv

Table of Contents .......................................................................................... v

List of Tables .................................................................................................. ix

List of Figures ................................................................................................ xii

List of Abbreviations ...................................................................................... xx

List of Schemes .............................................................................................. xxii

1 Introduction .................................................................................................. 1

   1.1 Methuosis-Inducing Small Molecule Discovery ....................................... 3

   1.2 α,β-Unsaturated Ketones and Michael Addition ...................................... 3

   1.3 MOFLIPP .............................................................................................. 8

   1.4 Metabolism ........................................................................................... 9

   1.5 Analogues ........................................................................................... 12

2 Michael Addition ......................................................................................... 14

   2.1 Introduction .......................................................................................... 14

   2.2 Reaction Conditions ............................................................................. 18
2.3 HPLC/LCMS

2.3.1 HPLC Method and Internal Standard

2.3.2 Compound 7 and 27

2.3.3 Compound 8 and 28

2.3.4 Compound 9 and 29

2.3.5 Compound 10

2.3.6 Compound 11

2.3.7 Compound 12

2.3.8 Compound 13

2.3.9 Compound 14

2.3.10 Compound 15

2.3.11 Compound 16

2.3.12 Compound 17

2.3.13 Compound 18

2.3.14 Compound 19

2.3.15 Compound 20

2.3.16 Compound 21

2.3.17 Compound 1
2.3.18 Compound 2 ..................................................135
2.3.19 Compound 22 ..................................................143
2.3.20 Compound 23 ..................................................148
2.3.21 Compound 24 ..................................................151
2.3.22 Compound 25 ..................................................156
2.3.23 Compound 26 ..................................................164
2.3.24 Results Summary and Discussion ...............................................................169
2.4 Synthesis of Michael Acceptors .................................................................178
2.5 Conclusion .................................................................181
2.6 Experimental .................................................................182
   2.6.1 Materials and Methods .................................................................182
3 Metabolite Synthesis .................................................................192
   3.1 Introduction .................................................................192
   3.2 Reductions .................................................................194
      3.2.1 Results and Discussion .................................................................195
   3.3 Oxidation .................................................................197
      3.3.1 Results and Discussion .................................................................197
   3.4 Conclusion .................................................................198
4 Indole-Based Chalcone Analogues.............................................204

4.1 Introduction.............................................................................204

4.2 Synthesis of Phenyl Derivatives.............................................205

4.3 Biological Activity.................................................................208

4.4 Conclusion..............................................................................208

4.5 Experimental.........................................................................208

4.5.1 Materials and Method.........................................................208

References..................................................................................214

A Biological Activity Data............................................................216

B NMR Spectra for Synthesized Compounds.................................218
List of Tables

1.1 Historical and New Analogues on the Pyridinyl Ring........................................13

2.1 Michael Acceptor Functional Group Study..........................................................16

2.2 Reaction Conditions with Relative Reaction Rates..............................................19

2.3 HPLC Gradient Over 20 min..............................................................................22

2.4 Purity Percentage for 7......................................................................................24

2.5 HPLC Gradient Over 25 min..............................................................................24

2.6 Purity Percentage for 27....................................................................................27

2.7 Purity Percentage for 8......................................................................................35

2.8 Purity Percentage for 28....................................................................................37

2.9 Purity Percentage for 9......................................................................................42

2.10 Purity Percentage for 29..................................................................................44

2.11 Purity Percentage for 10..................................................................................49

2.12 Purity Percentage for 11..................................................................................55

2.13 Purity Percentage for 12..................................................................................63

2.14 Purity Percentage for 13..................................................................................71

2.15 Purity Percentage for 14..................................................................................79
2.16 Purity Percentage for 15 .................................................................87
2.17 Purity Percentage for 16 .................................................................94
2.18 Purity Percentage for 17 .................................................................102
2.19 Purity Percentage for 18 .................................................................111
2.20 Purity Percentage for 19 .................................................................119
2.21 Purity Percentage for 20 .................................................................124
2.22 Purity Percentage for 21 .................................................................127
2.23 Purity Percentage for 1 .................................................................130
2.24 Purity Percentage for 2 .................................................................135
2.25 Purity Percentage for 22 .................................................................143
2.26 Purity Percentage for 23 .................................................................148
2.27 Purity Percentage for 24 .................................................................151
2.28 Purity Percentage for 25 .................................................................156
2.29 Purity Percentage for 26 .................................................................164
2.30 Michael Addition Reactivity .......................................................171
2.31 Reactant and Michael Product Parent compound and Fragments ............173
2.32 Final Compound and Starting Material with Corresponding Method ..........179
3.1 Reduction Reagent and Product for Standard .....................................195

x
3.2 Reducing Reagent and Product for MOMIPP ........................................196
4.1 Summary of Phenyl Analogues ..............................................................205
# List of Figures

1-1 Artist Rendition of the Methuotic Phenotype Observed in Glioblastoma Cells Upon Treatment with Certain Drugs ................................................................. 2

1-2 Structure of Methuosis Lead Compound Called MOMIPP and Another Compound of Interest Called MOFLIPP ................................................................. 3

1-3 Alpha-beta Unsaturated Ketones Present in Drug-Like Molecules which are Either Marketed and/or Found to be Michael Acceptors t Cysteine Residues ................. 6

1-4 Overall Reactivity of Michael Acceptors having Adjacent Heteroatoms .............. 8

1-5 Amides Enolate-Like Formation on a Michael Acceptor ................................. 8

1-6 MOMIPP Metabolite Formation and Prediction ............................................. 10

2-1 Michael Donor and Acceptor Standard .......................................................... 15

2-2 Internal Standard ............................................................................................ 18

2-3 Standard Concentration Curve for Naphthalene ......................................... 22

2-4 Peak Absorption and Chromatogram for 7 ................................................... 24

2-5 Calibration Chromatogram and Curve for 7 ................................................ 26

2-6 Peak Absorption and Chromatogram for 27 ................................................ 27

2-7 Calibration Chromatogram and Curve for 27 ............................................. 28
2-62 LCMS SIR for 15 Michael Addition Reaction ..................................................93
2-63 Peak Absorption and Chromatogram for 16 .....................................................94
2-64 Calibration Chromatogram and Curve for 16 ..................................................95
2-65 Change in 16 During the Michael Addition Reaction ......................................96
2-66 The Percentage of Michael Product Formed Over Time ..................................98
2-67 Percent of Remaining Michael Product Formed Over Time ............................99
2-68 Optimal Wavelength for Michael Addition Product Formed ............................99
2-69 LCMS SIR for 16 Michael Addition Reaction ..................................................100
2-70 LCMS/MS Daughter Scan for 16 .................................................................101
2-71 Peak Absorption and Chromatogram for 17 .....................................................102
2-72 Calibration Chromatogram and Curve for 17 ..................................................103
2-73 Change in 17 During the Michael Addition Reaction .................................105
2-74 The Percentage of Michael Product Formed Over Time ..............................107
2-75 Percent of Remaining Michael Product to be Formed .................................108
2-76 Optimal Wavelength for Michael Addition Product Formed for 17 .............108
2-77 LCMS SIR for 17 Michael Addition Reaction ..................................................109
2-78 LCMS/MS Daughter Scan for 17 .................................................................110
2-79 Peak Absorption and Chromatogram for 18 .................................................111
Calibration Chromatogram and Curve for 18

Change in 18 During the Michael Addition Reaction

The Percentage of Michael Product Formed Over Time

Percent of Remaining Michael Product to be Formed

Optimal Wavelength for Michael Addition Product Formed from 18

LCMS SIR for 18 Michael Addition Reaction

LCMS/MS Daughter Scan for 18

Peak Absorption and Chromatogram for 19

Calibration Chromatogram and Curve for 19

Change in 19 During the Michael Addition Reaction

LCMS SIR for 19 Michael Addition Reaction

Peak Absorption and Chromatogram for 20

Calibration Chromatogram and Curve for 20

Change in 20 During the Michael Addition Reaction

Peak Absorption and Chromatogram for 21

Calibration Chromatogram and Curve for 21

Change in 21 During the Michael Addition Reaction

Peak Absorption and Chromatogram for 1
2-116 Peak Absorption and Chromatogram for 24……………………………………151
2-117 Calibration Chromatogram and Curve for 24……………………………………152
2-118 Change in 24 During the Michael Addition Reaction…………………………..153
2-119 LCMS SIR for 24 Michael Addition Reaction…………………………………155
2-120 Peak Absorption and Chromatogram for 25……………………………………156
2-121 Calibration Chromatogram and Curve for 25……………………………………157
2-122 Change in 25 During the Michael Addition Reaction…………………………..158
2-123 The Percentage of Michael Product Formed Over Time…………………………160
2-124 Percent of Remaining Michael Product to be Formed…………………………..161
2-125 Optimal Wavelength for Michael Addition Product Formed from 25…………161
2-126 LCMS SIR for 25 Michael Addition Reaction…………………………………163
2-127 Peak Absorption and Chromatogram for 26……………………………………164
2-128 Calibration Chromatogram and Curve for 26……………………………………165
2-129 Change in 26 During the Michael Addition Reaction…………………………..167
2-130 LCMS SIR for 26 Michael Addition Reaction…………………………………168
3-1 MOMIPP Parent Molecule and Metabolites………………………………………193
3-2 Synthetic Strategies for Synthesis of MOMIPP Metabolites………………………194
List of Abbreviations

µL ...................... microliter
µM ...................... micromolar
°C ...................... degrees Celsius

ACN .................. Acetonitrile
calcd .................. calculated
CDCl₃ .................. Deuterated chloroform
¹³C NMR .............. carbon nuclear magnetic resonance
d ...................... doublet
dd ..................... double of doublets
DCM .................. Dicloromethane
DMF .................. N, N-Dimethyl formamide
DMSO ................ dimethyl sulfoxide

Et₃N, TEA ............. triethylamine
EtOAc ................ ethyl acetate
EtOH .................. ethanol
eq ..................... equivalent

g ...................... gram
g/mol .................. grams/mole
GI₅₀ .................. growth inhibition of 50%

h ...................... hours
H₂…………………hydrogen
HCl…………………hydrochloric acid
¹H NMR……………proton nuclear magnetic resonance
H₂O…………………water
HPLC………………high pressure liquid chromatography
Hz…………………hertz

LAH………………Lithium Aluminum Hydride
LCMS………………liquid chromatography mass spectrometer

m…………………multiplet
MeOH………………methanol
mg…………………milligram
MHz………………megahertz
min………………minute(s)
MIPPP………………3-(2-methyl-1H-indol-3-yl)-(4-pyridinyl)-2-propan-1-one
ml………………milliliters
mmol………………millimole
MOFLIPP…………3-(5-methoxy-2-trifluoromethyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propan-1-one
MOMIPP…………3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one
mp…………………melting point

N…………………normal

PAIS………………peak area of the internal standard
PAP…………………peak area of the product
PAR…………………peak area of the reactant
PBS………………phosphate buffered saline solution
PDA………………Photodiode Array
PDC..................pyridium dichromate
Pd/C..............palladium over carbon
POCl₃..............phosphorus oxychloride

rt....................room temperature
s...........................singlet
SAR.....................Structure-Activity Relationship

t..........................triplet
TFA....................trifluoroactic acid
THF...................tertahydrofuran
TLC...................thin-layer chromatography

UV......................ultraviolet
List of Schemes

1-1 General Michael Addition Reaction with the Nucleophile Represented as H-Nu:....5

2-1 Michael Addition Reaction with Standards.................................................19

2-2 Michael Addition Reaction with Standard...............................................20

2-3 Synthesis of 4[(Phenylmethyl)thio]-2-butanone (27) and 4-Thiobenzyl-2-pentanone (28) and 4-Phenyl-4-(phenylthio)-2-butanone (29).................................182

2-4 General Synthesis for Various Chalcones. ....................................................182

2-5 Synthesis of 3-(5-methoxy, 2-methyl-1H-indol-3-yl)-1-(4-benzyl)-2-propen-1-one..............................................................................................................184

2-6 Synthesis of 3-(5-methoxy, 2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one..............................................................................................................184

2-7 Synthesis of 3-Diphenyl-2-propenamide.........................................................185

4-1 Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(acetylaminophenyl)-2-propen-1-one..............................................................................................................210
4-2 Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(N-boc-aminophenyl)-2-propen-1-one and 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1(aminophenyl)-2-propen-1-one.................211

4-3 Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(nitrophenyl-2-propen-1-one...211
Chapter 1

Introduction

Programed cell death, called “apoptosis”, has been studied for over a century. This well-defined process is characterized by plasma membrane blebbing, cytoplasm shrinkage, nuclear chromatin condensation and the formation of apoptotic bodies. Many anticancer drugs take advantage of this fundamental pathway to kill cancer cells. Subsequent to the identification of apoptosis, several other types of cell death have been discovered and classified. Recently, a novel, non-apoptotic type of cell death, which is referred to as “methuosis”, has been identified phenotypically as the formation and accumulation of vacuoles arising from faulty cell trafficking, leading to rupture of the cell membrane. It is known to be caspase-independent whereas apoptosis is caspase-dependent. An artist’s rendition of the methuotic process as observed in glioblastoma cells is depicted in Figure 1-1. However, the mechanism for cell death by which methuosis operates is still unknown.
The initial report on methuosis studied human glioblastoma and gastric carcinoma cells which were mutated with oncogenic Ras gene. It was observed that cell death occurred after vacuoles formed and swelled leaving the nuclei relatively unaffected. It was later identified that the vacuoles originated from macropinosomes. Macropinosomes form when mammalian cells take in extracellular fluid and nutrients to form vacuoles. These vacuoles can either fuse with a lysosome or recycle back to the extracellular fluid. In the glioblastoma cells over-expressed with the mutated Ras gene, macropinocytosis is stimulated but dysfunctional. Instead of recycling or fusing with lysosomes, these vacuoles accumulate, perturb cellular function and ultimately rupture the cell membrane. During this process leading to cell death there is no sign of shrinkage, chromatin condensation nor plasma membrane blebbing which is seen during apoptosis.⁴
1.1 Methuosis-Inducing Small Molecule Discovery

Screening for small molecules that might trigger this novel form of cell death, the indolyl-pyridinyl-propenone \(3-(5\text{-methoxy}-2\text{-methyl}-1H\text{-indol}-3\text{-yl})-1-(4\text{-pyridinyl})-2\text{-propen}-1\text{-one}\) (MOMIPP, compound 1 in Figure 1-2) was identified as a methuosis-inducer at low micromolar concentration.\(^5\) Drug-resistant cancers contain mutations in genes that can circumvent an efficient apoptotic response. Thus, methuosis-inducing compounds are of interest because they may provide a new drug therapy paradigm that relies on non-apoptotic cell death to address current drug-resistant cancers.

![Figure 1-2. Structure of Methuosis Lead Compound called MOMIPP and Another Compound of Interest called MOFLIPP. For MOMIPP (compound 1) \(R^1=\text{OCH}_3\) and \(R^2=\text{CH}_3\). For MOFLIPP (compound 2) \(R^1=\text{OCH}_3\) and \(R^2=\text{CF}_3\).]

1.2 \(\alpha, \beta\)-Unsaturated Ketones and Michael Additions

Structure activity relationship (SAR) studies have been conducted and define structural features on the indolyl and pyridinyl rings which are required for methuosis induction.\(^5,6,7\) In addition to investigating the \(R^1\) and \(R^2\) substituents (Figure 1-2), modifications have been made on the pyridinyl moiety. The central region remains unexplored in terms of the SAR. This region contains an \(\alpha, \beta\)-unsaturated ketone which
generally are thought to be candidates for Michael addition reactions. A Michael addition reaction typically involves a base-catalyzed, nucleophilic addition of an enolate anion (Michael donor) to an activated α,β-unsaturated carbonyl compound (Michael acceptor). However, non-carbon nucleophiles are now also recognized as Michael donors as there can be carbon-Michael addition, oxa-Michael addition, aza-Michael addition and thiol-Michael addition amongst others.\(^9\) From these possibilities, the thiol-Michael addition was chosen for our focus due to its biological significance and greater nucleophilicity when compared to amines. Overall, the Michael addition reaction mechanism is the same regardless of the nucleophile, e.g. the nucleophile is deprotonated which then attacks the \textit{beta}-carbon causing a flow of electrons, moving the carbon-carbon double bond and subsequently leaving a negative charge on oxygen. This high-energy negatively charged species will collapse back to the ketone causing protonation on the \textit{alpha}-carbon yielding a 1,4-addition as depicted in Scheme 1-1.\(^8,9\) The 1,4-addition at the \textit{beta}-carbon can occur readily in the presence of a strong nucleophile such as thiols.\(^9\) Under certain conditions, a 1,2-addition can occur. However, the latter will not be further discussed given our intended focus on the use of a strong nucleophile which mimics the presence of thiols in glutathione as well as cysteine residues found in many proteins.
Scheme 1-1. General Michael Addition Reaction with the Nucleophile Represented as H-Nu:.

While the first step can be reversible as indicated by the dual half-arrows, the second step is generally considered to be irreversible (see text for examples and potential exceptions). Electron arrows are depicted only for progression toward final product.

There are several examples of drug-like molecules and even some marketed drugs which utilize Michael addition on an α,β-unsaturated ketone for protein inhibition.\textsuperscript{10,11} For example, covalent, irreversible kinase inhibitors bind to an exposed cysteine residue blocking the ATP binding site and rendering the kinase inactive.\textsuperscript{10} Figure 1-3 illustrates several of the drugs marketed in the U.S. which contain an α,β unsaturated ketone, some of which have been shown to undergo a Michael addition reaction.
Figure 1-3. Alpha-beta Unsaturated Ketones Present in Drug-like Molecules which are either Marketed and/or Found to be Michael Acceptors to Cysteine Residues. Dutasteride (Avodart) marked with the asterisk was number 81 in the top 200 best-selling drugs in 2010, marketed as a 5-alpha-reductase inhibitor to treat enlarged prostate. Even though there is an unhindered α,β unsaturated ketone, the drug is reduced via NADH and testosterone reductase. There is no evidence that this compound would undergo Michael addition. Afatinib, however, is a marketed drug which irreversibly inhibits EGFR tyrosine kinase at the cysteine residue, exemplifying a Michael addition reaction. The remaining compounds are irreversible kinase inhibitors with HKI-272 specifically being used for targeting specifically positioned cysteine residues.

Notably, all of the kinase inhibitors and the one indicated by an asterisk in Figure 1-3 have accessible α,β unsaturated ketone systems. Alternatively, this feature is rather encumbered within the steric features of MOMIPP’s scaffold. Thus, it remains questionable whether or not MOMIPP is a candidate for a Michael addition reaction. Adding to the complexity of this question is to what effect will indolyl or pyridinyl
functionalities have on the Michael acceptor capability. The influence of these heterocycles is not delineated in the literature.

Electron withdrawing groups can enhance the Michael acceptor\(^8\) by causing the olefin to be more electron deficient. Electron donating groups will cause the olefin to be more electron rich and decrease reactivity. Figure 1-4 illustrates the relative reactivity of various Michael acceptors. Electron donating groups lower reactivity when compared to other Michael acceptors.\(^8\) While this figure summarizes known literature, it does not describe how various heterocyclic groups effect the overall reactivity of the Michael acceptor. The nature of the specific heterocycles present in MOMIPP and their role in regard to possible Michael addition reactions remain to be determined. Interestingly, the amides that are found in most of the compounds in Figure 1-3 can stabilize the enolate-like compound depicted in Figure 1-5. This enolate-like compound does not prevent a Michael addition but the overall reactivity of the Michael acceptor is much lower than if there was no amide. Thus, for the drug-like molecules in Figure 1-3, the amide may be needed for more selective interactions with the surrounding amino acid residues found in the active site of the target enzyme rather than for having general or promiscuous reactivity at off-target sites that could cause unwanted side-effects and toxicity. Finally, in some cases Michael addition reactions can also be reversible depending on the structure of the Michael acceptor and the attacking donor as well as on the pH and polarity of the solvent.\(^13\) All of these variables contribute to the overall reaction outcome in the specific cases for any given molecule.
Figure 1-4. Overall Reactivity of Michael Acceptors having Adjacent Heteroatoms. Amides and esters have lower reactivity due to their electron donating characteristics.\(^8\) The latter is shown for amides in the accompanying Figure 5.

![Diagram showing reactivity of amides and esters with heteroatoms]

Figure 1-5. Amide Enolate-like Formation on a Michael Acceptor. The lone pair of electrons on the nitrogen will drift toward the carbonyl causing a partial negative charge on the oxygen as well as a partial positive charge on the nitrogen. Due to this movement it allows for the electrons to be shared between the oxygen and nitrogen indicated by the dotted line between the two in the above illustration.

1.3 MOFLIPP

Another notable compound identified from previous SAR studies was 3-(5-methoxy-2-trifluoromethyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (MOFLIPP, compound 2 in Figure 1-2) which was cytotoxic in cell-based assays at sub-micromolar concentration. However, MOFLIPP forms little to no vacuoles. This remarkable difference in biological activity between such closely related chemical structures suggest that these two compounds interact with different cellular pathways. A trifluoromethyl group would be an example of a strong electron withdrawing group as compared to the corresponding methyl group on MOMIPP (1). The effect of this electron withdrawal on the \(\alpha,\beta\)-unsaturated ketone and, in turn, its willingness to participate in Michael addition reactions when compared to MOMIPP could contribute to the difference in cell-death mechanisms.
Thus, the impact of the electronics not only on the potential Michael addition reactivity, but also how that relates to the possible different mechanism of action between these two compounds, becomes an intriguing question to address.

**1.4 Metabolism**

When constructing drug-like compounds, metabolism becomes an important consideration in determining the suitability of a molecule as a potential drug. The metabolism of MOMIPP when cultured with human U251 glioblastoma cells was ascertained within our Center for Drug Design and Development (CD3). MOMIPP was added to media and cultured for 4, 24, 48, or 72 hours with and without U251 cells.\(^1\)

Figure 1-6 Panel A illustrates the HPLC-MS analyses of U251 cell cultures incubated with MOMIPP showing three metabolites later defined in Figure 1-6 Panel D. The mass spectrum (Figure 1-6 Panel B) shows that the experimental mass for MOMIPP was 293.1 compared to the theoretical exact mass for MOMIPP of 293.13 giving a positive identification of unmetabolized MOMIPP. Three other peaks were found, one major and one minor at 295.1 (M2B and M2A, respectively), representing MOMIPP plus two hydrogens, and one at 297.1(M1), representing MOMIPP plus four hydrogens. This indicates that first M2B (and smaller amount of M2A) is formed from MOMIPP, and then M1 is formed from M2B or M2A. The daughter ionization shows that MOMIPP has a

---

\(^1\) Samples were analyzed via 10µL injections onto a Waters Ascentis Express C18 column, 75 x 21 µM, with matching guard column. An isocratic mobile phase containing water (0.1% formic acid) and acetonitrile (ACN) was adjusted between 20-50% ACN to give a 5-6 min run at 0.3 mL/min flow rate using a Waters 2795 HT-Alliance LC Separations Module. Detection was accomplished via a Micromass Quattro Micro API Detector with Micromass MassLynx v4.1 software. SIR/Parent MS – ES+, Cone V 25, Cap kV 3.0 and MRM/Daughter MS/MS – ES+, Cone V 30, Collision V 28, Source 100°C, Desolation 400°C/650 L/hr.14,15
complex ionization pattern and M1 and M2B have a very simple daughter ionization pattern with two primary ions near 122 and 174 g/mol (Figure 1-6 Panel C). Figure 1-6 Panel D shows the possible metabolites formed from MOMIPP. Each provides the observed parent and daughter ions even though there are other possible metabolites. However, M2B would elute after MOMIPP and M2A and M1 would elute before MOMIPP based on polarity of the compounds.\textsuperscript{14,15}
Figure 1-6. MOMIPP Metabolite Formation and Prediction. A) HPLC analysis of MOMIPP standard, 24, 48, and 72 h (MOMIPP with cells, cells with no MOMIPP, and MOMIPP with no cells). B) Ionization of parent compound for MOMIPP, M1, and M2. C) Daughter ionization pattern for MOMIPP, M1, and M2 which demonstrates the complex ionization for MOMIPP and simple ionization pattern for M1 and M. D) The metabolism pathway for MOMIPP with M2 divided into M2A and M2B both partially reduced versions of MOMIPP. Both M2A and M2B will be converted into M1 which is the fully reduced version of MOMIPP.

The synthesis of the metabolites of MOMIPP have been attempted with the successful synthesis of **M1** as a part of this thesis. The metabolites formed are reduced forms of MOMIPP, M2 being partially reduced and M1 fully reduced versions of MOMIPP. There are several ways to potentially synthesize these compounds using various
reducing reagents such as sodium borohydride, lithium aluminum hydride, catalytic reductions (e.g. Pd/C) under H$_2$ atmosphere being the more common, or even enzymes to preform reductions of olefins and ketones. Another avenue to achieve the partially reduced, M2B metabolite (Figure 1-6D), is oxidation of the fully reduced metabolite, with various oxidants such as pyridinium chlorochromate, pyridinium dichromate, and Dess Martin. All of these possible reactions will be discussed in more detail in a later chapter.

1.5 Analogues

The SAR studies previously have been performed with modifications on the indolyl and pyridinyl moieties. Substitutions on the indolyl moiety have been extensively studied,$^{5,6,7}$ however, less structural information is known for the pyridine functionality. Substitutions containing ortho- and meta- pyridine as well as pyrazine, and phenyl have been synthesized and evaluated. All were found to be inactive.$^6$ This led to the conclusion that when having a N-containing heterocycle, placing the nitrogen at the para-position of the pyridine ring was essential for methuosis-induction.$^6$ Alternatively, the effect on activity when the nitrogen is placed external to the ring is not known. Four of such analogues have been synthesized as part of this thesis project. They encompass a range of electronic effects and have been tested for their ability to induce methuosis. These derivatives include: an acylated and a boc-protected amine; a free amine; and a nitro analogue. The nitro analogue is the most electron-withdrawing followed by the acylated, boc-protected and then the free amine as the most electron donating moiety. The historical and new analogs are shown below in Table 1.1.
Table 1-1. **Historical and New Analogues on the Pyridinyl Ring.** Compounds in column 1 are historical analogues with the activity listed in column 2. Compounds in column 3 are new analogs prepared as part of this thesis and their activities are discussed in chapter 4.

![Pyridinyl Ring](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Activity</th>
<th>R</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Pyridine" /></td>
<td>No</td>
<td><img src="image" alt="Pyridine with Carboxyl" /></td>
<td>Discussed Later</td>
</tr>
<tr>
<td><img src="image" alt="Pyridine" /></td>
<td>No</td>
<td><img src="image" alt="Pyridine with Carboxyl" /></td>
<td>Discussed Later</td>
</tr>
<tr>
<td><img src="image" alt="Pyridine" /></td>
<td>No</td>
<td><img src="image" alt="Pyridine with Ammonia" /></td>
<td>Discussed Later</td>
</tr>
<tr>
<td><img src="image" alt="Pyridine" /></td>
<td>Yes</td>
<td><img src="image" alt="Pyridine with Nitro" /></td>
<td>Discussed Later</td>
</tr>
</tbody>
</table>


Chapter 2

Michael addition

2.1 Introduction

As described in the previous chapter, Michael addition reactions occur by nucleophilic attack at the β position of an α,β unsaturated carbonyl systems (Michael acceptor system). This attack can occur by a number of nucleophiles (Michael donors) including carbanions, oxygens, nitrogens, and thiols. Some of these nucleophiles are present in biological systems. The lead methuosis-inducing compound called MOMIPP contains an α,β unsaturated ketone (Figure 2-1). Thus, there is a possibility for a Michael addition reaction within the biological environment and this could be relevant for either MOMIPP’s mode of desirable activity or its potential for off-target toxicity, all of which remains unknown. In this regard, it is unclear what role the heterocyclic groups present on either side of the α,β-unsaturated ketone in MOMIPP may play toward enhancing or prohibiting such reaction. There are some literature references\textsuperscript{8,9} that pertain to the influence of substitutions on the Michael acceptor but there is not enough information to determine if the heterocyclic groups present in MOMIPP would favor or disfavor Michael addition. Therefore, an investigation of these specific groups was needed to assist with
determining the potential for Michael addition so that this feature could be related to biological systems and potential drug modifications.

A general study was first conducted to determine appropriate conditions for the Michael addition reaction. Initially two Michael donor and two Michael acceptor standards were used. These Michael donors and acceptors are depicted in Figure 2-1. They were shown to readily undergo Michael addition reactions under a variety of conditions. In this study the overall reaction rate was a primary factor to be optimized. To allow for later comparison of the unknown substituents, it was decided that a moderate (several hours to a few days) reaction rate would be preferable. Too fast a rate could preclude analytical measurements and too slow a rate could prolong experiments past practicality. Three conditions were explored: MeOH with base, MeOH alone, and MeOH/buffer pH=7.4, the latter reflecting the biological setting.

![Michael Donor and Acceptor Standards](image)

**Figure 2-1. Michael Donor and Acceptor Standards.** For the Michael donors $R=\text{Ph}$ or $\text{CH}_2\text{Ph}$ and for the Michael acceptors $R_1=\text{H}$, $\text{CH}_3$, or $\text{Ph}$ and $R_2=\text{CH}_3$.

Once appropriate conditions were devised (Section 2.4.2) a series of compounds were explored. This series provided a transition of properties present in the substituents on the Michael acceptor that started with compounds that do not induce methuosis and then gradually led to the specific case of MOMIPP. Table 2.1 lists the progression of Michael acceptors while the Michael donor was held constant.
Table 2.1 Michael Acceptor Functional Group Study. An asterisk (*) indicates that the entry was made by synthesis as part of this study see Sections 2.5 for synthetic scheme and 2.6 for experimental. A double asterisk (**) indicated that the entry was made by other scientists on our project team as part of their structure-activity studies.

![Chemical Structure](image.png)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>H⁻</td>
<td>CH₃⁻</td>
</tr>
<tr>
<td>8</td>
<td>CH₃⁻</td>
<td>CH₃⁻</td>
</tr>
<tr>
<td>9</td>
<td>Ph⁻</td>
<td>CH₃⁻</td>
</tr>
<tr>
<td>10</td>
<td>CH₃⁻</td>
<td>Ph⁻</td>
</tr>
<tr>
<td>11⁺</td>
<td>Ph⁻</td>
<td></td>
</tr>
<tr>
<td>12⁺</td>
<td>Ph⁻</td>
<td><img src="image.png" alt="Structure" /></td>
</tr>
<tr>
<td>13⁺</td>
<td>Ph⁻</td>
<td><img src="image.png" alt="Structure" /></td>
</tr>
<tr>
<td>14</td>
<td><img src="image.png" alt="Structure" /></td>
<td>Ph⁻</td>
</tr>
<tr>
<td>15</td>
<td><img src="image.png" alt="Structure" /></td>
<td>Ph⁻</td>
</tr>
<tr>
<td>16⁺</td>
<td><img src="image.png" alt="Structure" /></td>
<td>Ph⁻</td>
</tr>
<tr>
<td>17⁺</td>
<td>Ph⁻</td>
<td><img src="image.png" alt="Structure" /></td>
</tr>
<tr>
<td>18⁺</td>
<td>Ph⁻</td>
<td><img src="image.png" alt="Structure" /></td>
</tr>
<tr>
<td>19⁺</td>
<td><img src="image.png" alt="Structure" /></td>
<td>Ph⁻</td>
</tr>
</tbody>
</table>
The method devised from the initial study of reaction conditions was further refined for use during the analytical characterization of reaction rate for all of the compounds represented in Table 2.1. From this list of compounds factors such as solubility, and chromatographic detection are of concern. For each compound a standard concentration curve was acquired to determine a linear response by HPLC analysis with UV detection.
From this aliquot dilution protocols were devised so that reaction mixtures were performed at a constant concentration. An internal standard, Figure 2-2, was also developed to account for evaporation of solvent during reactions or injection errors during analyses.

![Compound](image)

**Figure 2-2. Internal Standard.** Non-reactive internal standard, naphthalene. Its a large chromophore allowed for a low concentration within the reaction media.

After each calibration curve was derived, the Michael addition reaction test was started and aliquots were collected and measured 0, 1, 3, 6, 24, 28, 48, 54, 72, and 96 h. After 96 h, 1 eq. of trimethylamine (TEA) was added, with a final sample collected 1 h later. These analyses measured the disappearance of starting material with time. To determine product formation with time, either a calibration curve for the Michael product was made whenever the product was available, or reaction mixture samples were analyzed by LC-MS and LC-MS/MS to verify formation of the expected product. The thiol reactant was not monitored during the reaction due to the excess present during the reaction and because it is known to be sensitive toward autoxidation and deterioration.

### 2.2 Reaction conditions

Three sets of reaction conditions were tested to determine an overall rate compatible for later analytical analysis of a variety of test substances. Two Michael acceptors and Michael donors were reacted under these three sets of reaction conditions. Scheme 2-1 depicts the Michael acceptors paired with the Michael donors with the three
sets of reaction conditions listed in Table 2.2 along with the relative rate for each corresponding condition.

Scheme 2-1. Michael Addition Reaction with Standards. Two different Michael acceptors paired with two different Michael donors.

Table 2.2. Reaction Conditions with Relative Reaction Rates

<table>
<thead>
<tr>
<th>Reaction Condition</th>
<th>Relative reaction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH with 0.5 eq. trimethylamine</td>
<td>Minutes</td>
</tr>
<tr>
<td>MeOH no base</td>
<td>Days</td>
</tr>
<tr>
<td>50% MeOH 50% phosphate buffered saline solution (PBS) pH 7.4</td>
<td>Within 1 h</td>
</tr>
</tbody>
</table>

From the three set of reaction conditions, MeOH with base, straight MeOH, and MeOH-buffer, the four standard pairs reacted in MeOH with base reached completion within minutes. Thus these conditions allow for reactions that are too quick to be later adapted into an analytical method. The second set of condition, namely straight MeOH, provided reactions running one to two days. This reaction rate can be used in an analytical
The last reaction condition investigated was MeOH with PBS buffer and this lead to reaction rates of within 1 h. To account for solubility issues of some of the unknown compounds listed in Table 2.1, DCM was added to the MeOH without base at a ratio of 2:1 DCM:MeOH with little to no effect on the overall reaction rate.

While the standards deployed to determine overall reaction rate in the lab were useful, they do not have a strong chromophore for convenient UV detection in our subsequent HPLC studies. Therefore we also examined 9 as an additional standard. Our preliminary adaption of the HPLC/LCMS methods is discussed in the next section wherein this standard becomes highlighted. Scheme 2-2 depicts the synthesis of the Michael product of 7.

Scheme 2-2. Michael Addition Reaction with Standard. The Michael acceptor with a stronger chromophore was reacted with both Michael donors, R= Ph or CH\textsubscript{2}Ph, using MeOH and TEA to achieve product formation as proof of reactivity as well as to be used later as a standard for HPLC analysis. To adopt this reaction method of straight MeOH into a condition which can be used for HPLC analysis and be applicable with future compounds, the reaction condition was modified to a mixture of DCM and MeOH at a 2:1 ratio respectively. The reactivity does not change significantly and still leads to Michael addition product.
2.3 HPLC/LCMS

2.3.1 HPLC Method and Internal Standard

HPLC analysis was performed using an Alliance® HPLC (model Waters 2659) equipped with a quaternary pump, an inline membrane degasser, an autosampler and a Waters Corporation (Milford, MA, USA) column oven. Detection was conducted with a Waters® 2996 Photodiode Array (PDA) Detector. Data acquisition and processing utilized from Empower software from Waters. All samples were analyzed using a Nova-Pak®C18 analytical column (reverse-phase, 3.9x150 mm, 4µm) purchased from Waters Corporation (Milford, MA, USA). It utilized 10 µL injections of test sample. The flow rate was 1 mL/min across the gradient delineated in Table 2.3. For each calibration curve, the Michael acceptor is treated as the reaction mixture, with a specific amount of acceptor dissolved in DCM/MeOH (2:1). A calculated amount is removed and then diluted with acetonitrile (ACN) and a second time with ACN: 1 M PBS buffer pH=7.4 2:3 to four concentrations, 5 µM, 10 µM, 15 µM, and 20 µM with an n=3 to generate the calibration curve. For the internal standard the calibration curve was created with the concentrations 1 µM, 2.5 µM, 5 µM, and 7.5 µM, depicted below in Figure2-3.
Table 2.3. HPLC Gradient Over 20 min. For the curve column, the number 6 denotes a linear increase in percentage of mobile phase over time while number 1 denotes an immediate change in mobile phase.

<table>
<thead>
<tr>
<th>Time</th>
<th>Water%</th>
<th>ACN%</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2.00</td>
<td>45</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>15.00</td>
<td>20</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>15.01</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>20.00</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2-3. Standard Concentration Curve for Naphthalene. Chromatogram for naphthalene at 1, 2.5, 5 and 7.5 μM. Notice the change in scale of the y axis (absorption) amongst the four concentrations. Standard concentration curve for naphthalene with n = 3 comparing the peak area against solution concentration. Each sample was treated as the Michael addition reactions: dissolve in DCM:MeOH (2:1), then diluted twice with the final dilution at (2:3) ACN:PBS.
The calibration curve for the internal standard is linear. Any concentrations within this range could be used as the internal standard concentration. However, a low concentration was desired so as to minimize any effect on the Michael addition reaction rate. From this data, 2.5 µM was chosen for the internal standard concentration.

2.3.2 Compound 7 and 27

For each compound listed on Table 2.1, a number of chromatograms and data was collected: purity and optimal wavelength data, calibration curve and Michael addition reaction. For this compound, the HPLC method required change in order to separate the product peak from the thiol peak. The new method is illustrated in Table 2.5.

Figure 2-4 and Table 2.4 depicts compound 7, a commercial material, to have a purity of 94% at an optimal wavelength at 210 nm. This wavelength was used for the remainder of the experiments tracking the peak with a retention time of 1.78 min.
Figure 2-4. Peak Absorption and Chromatogram for 7. The peak absorption for 7 occurs at 210nm. This is the wavelength that was used for Michael addition tests with 7.

Table 2.4. Purity Percentage for 7. The purity of compound 7 was assessed at 210 nm at a concentration of 20 µM

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.78</td>
<td>139740</td>
<td>94</td>
</tr>
<tr>
<td>6.37</td>
<td>9040</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2.5. HPLC Gradient Over 25 min.

<table>
<thead>
<tr>
<th>Time</th>
<th>Water%</th>
<th>ACN%</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2.00</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>15.00</td>
<td>20</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>20.00</td>
<td>20</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>20.01</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>25.00</td>
<td>80</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>
The calibration curve was generated with an $n = 3$ at each concentration and related the ratio of the peak area of the reactant (PAR; $\mathbf{7}$) over the peak area of the internal standard (PAIS) to the known concentration. The slope of the graph generated from the calibration curve was then used for the reaction to determine the concentration throughout the reaction. This process was continued for each compound studied. Figure 2-5 depicts the chromatograms of compound $\mathbf{7}$ (210 nm) and the internal standard (219 nm).
Figure 2-5. Calibration Chromatogram and Curve for 7. Calibration curve for compound 7 with concentrations of 5, 10, 15, and 20 µM. Compound 7 was tracked at 210 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve reflects concentration vs the ratio of the peak area of 7 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.
Compound 7 was the first of three standards which were used to begin the study. The Michael addition product of 7 was generated in the previous discussed methods in 2.2 Reaction Conditions, Scheme 2.1. Compound 27 was formed as a Michael addition product standard. Figure 2-6 shows the optimal wavelength for compound 27 as well as its purity. It was synthesized with a purity of 91% and an optimal wavelength of 253 nm and a retention time of 9.23 min. Figure 2-7 illustrates the chromatogram for the calibration curve for 27. The internal standard was tracked at 219 nm while 27 was followed at 253 nm.

![Figure 2-6. Peak Absorption and Chromatogram for 27.](image)

The peak absorption for 27 occurs at 253 nm. This is the wavelength that was used for Michael addition tests with 27.

### Table 2.6. Purity Percentage for 27

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (Au)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.23</td>
<td>99084</td>
<td>91</td>
</tr>
<tr>
<td>10.99</td>
<td>9592</td>
<td>9</td>
</tr>
</tbody>
</table>
Figure 2-7. Calibration Chromatogram and Curve for 27. Calibration curve for compound 27 with concentrations of 5, 10, 15, and 20 µM. Compound 27 was tracked at 253 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve is concentration vs the ratio of the peak area of product, 27 (PAP) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction test.
For each Michael addition reaction conducted, the reaction was ran for a total of 97 h with an \( n = 4 \). At time point 0 h, there was no thiol present (reaction mixture only contains the reactant and internal standard). After the time point 0 h was collected, the thiol was added. Another sample was collected at time point 1 h. The amount of reaction mixture removed at 0 h and the thiol addition were very small compared to the total volume of each experiment, therefore, there was little change in overall concentration of reaction mixture.

For the first 12 h, a sample was removed and analyzed by HPLC every 6 hours in order to observe any fast acting reactions. Following 12 h, samples were analyzed every 12 h until 72 h. Following 72 h, two more samples were collected, one at 96 h and one at 97 h. After the 96 h sample was collected, 0.1 eq. of TEA was added and stirred for an additional hour, at which time the last sample was studied. This was done to try to enhance any Michael addition reactivity for those cases which did not react or had slow reactions. During the course of the comparative study conditions. This last time point was not considered for calculation of the rate constant. It was done as a verification that little or no reaction would occur for some types of substituted \( \alpha,\beta \)-unsaturated ketones.

Figure 2-8 illustrates the chromatograms for the Michael addition reaction with 7. The set of chromatograms at 210 nm shows the disappearance of 7 while those at 253 nm show the appearance of 7’s product, namely 27. The middle chromatograms at 219 nm illustrate the internal standard remaining constant. This case is distinct when compared to the other Michael acceptors due to the reaction being so quick that it goes to completion nearly immediately. The rate constant was calculated using the early data with the 1 h time point assigned a value of 1% of the initial concentration of the reaction so as to allow for a calculation of a rate constant being at least that fast.
Figure 2-8 Change in $[7]$ and $[27]$ During the Michael Addition Reaction. Reactant $7$ at 210 nm, internal standard at 219 nm, and Michael product $27$ at 253 nm. Chromatograms are represented at time points 0, 1, 24, 48, 72, 96 h. On the graph, the squares follow the reactant ($7$) while the circles follow the product ($27$).

One of the standard Michael addition reaction pairs was used to help develop a general LCMS and LCMS/MS\(^2\) method to verify Michael addition product formation. A single ion (SIR) method, which identifies the compound with the set mass, was used since the samples were at lower concentrations, a mass scan could not efficiently detect any reactant or product. The exact mass of the reactant and the product was used to predict the optimal ionized mass to generate a personalized SIR for each reaction.

\(^2\)Samples were analyzed \textit{via} 10 µL injections onto a Waters Ascentis Express C18 column, 75 x 21 µM, with matching guard column. An isocratic mobile phase modified for each compound using water (0.1% formic acid) and ACN to give a 5-10 min run at 0.3 mL/min flow rate using a Waters 2795 HT-Alliance LC Separations Module. Detection was accomplished \textit{via} a Micromass Quattro Micro API Detector with Micromass MassLynx v4.1 software. SIR/Parent MS – ES+, Cone V 25, Cap kV 3.0 and MRM/Daughter MS/MS – ES+, Cone V 25, Collision V 28, Source 100 °C, Desolation 400 °C/650 L/h.\(^{14,15}\)
Figure 2-9 depicts the LCMS data for 7 and the reaction at time point 96 h. Compound 7 was unable to be seen. This could be from ionization or concentration complications. However, the product, 27, was able to be seen at 2.84 min with a mass of 181.1 g/mol. This confirmed the practicality of deploying LCMS as method to verify product formation. As a result, it was not necessary to synthesize pure standard compound materials for each product so as to identify their peak locations on the LC chromatograms. Four set of masses were detected, the mass associated with the thiol (Figure 2-9 D), the mass associated with the reactant (Figure 2-9 C, 71.0 g/mol), the mass associated with the product (Figure 2-9 B, 181.1 g/mol), and the mass associated with the Michael intermediate (Figure 2-9 A, 180.1 g/mol). All mass detection was done in the positive mode with the exception of the Michael intermediate which was conducted in the negative mode. The Michael intermediate was not detected in any of the reactions therefore an example is shown below in Figure 2-9 A and will not be shown in the remaining chromatograms.
Figure 2-9. LCMS SIR for 7 and 27. A) The Michael intermediate was detected in the negative mode with a mass of 180.1 g/mol. B) Michael product 27 was detected in the positive mode with a mass of 181.1 g/mol. C) The reactant 7 was detected in the positive mode with a mass of 71.0 g/mol. D) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the reactant 7 and naphthalene. The chromatograms on the right was the 96 h inject which contains only Michael addition product 27 and Michael donor, thiophenol.

To further verify that the Michael addition product was formed rather than an arbitrary compound with the same parent mass, a daughter scan was performed to determine the fragments. After the fragment mass was found, ChemDraw was used to estimate the fragments. Table 2.31 lists all of the compounds with LCMS/MS data and the possible fragments to form the masses found.

Figure 2-10 illustrates the daughter ions for compound 27. Compound 7 did not show any fragments, this could be due to the parent molecule having such a low mass (70.0 g/mol). Any fragments formed would be too difficult to determine from the background. Therefore, there are only fragments and daughter ion scan for the Michael product 27.
major daughter ion found was 122.98 g/mol. The possible fragments with this mass can be seen in Table 2-31.

Figure 2-10. Daughter Scan for 96 h for 27. The major mass found at time point 2.84 min was approximately 123 g/mol. The structure of the possible fragments at this mass is listed in Table 2-31.
2.3.3 Compound 8 and 28

Figure 2-11 depicts compound 8’s optimal wavelength of 224 nm with a retention time of 2.31 min. This compound was commercially purchased and was found to have an approximate purity of 87%.

![Figure 2-11. Peak Absorption and Chromatogram for 8.](image)

The peak absorption for 8 occurs at 224 nm. This is the wavelength that was used for Michael addition tests for 8.

Table 2.7. Purity Percentage for 8. The purity of compound 8 was assessed at 224 nm at a concentration of 20 \( \mu \text{M} \).

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.31</td>
<td>65442</td>
<td>87</td>
</tr>
<tr>
<td>3.51</td>
<td>9516</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 2-12 shows the chromatograms and calibration curve for 8. The ratio of PAR/PAIS was correlated with the known concentration to be used for the Michael addition reaction.
Figure 2-12. Calibration Chromatogram and Curve for 8. Calibration curve for compound 8 with concentrations of 5, 10, 15, and 20 µM. Compound 8 was tracked at 224 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve represents concentration vs the ratio of the peak area of 8 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.
Compound 28 was another Michael addition product standard that was synthesized. This compound was accomplished with 88% purity when assessed at an optimal wavelength of 257 nm. Its retention time was 6.45 min (Figure 2-13 and Table 2.8). Its general synthesis can be seen in Scheme 2-1. The synthesis and experimental can be found in section 2.4 and 2.6 respectively.

Figure 2-13. Peak Absorption and Chromatogram for 28. The peak absorption for 28 occurs at 257 nm. This is the wavelength that was used for Michael addition tests for 28.

Table 2.8. Purity Percentage for 28. The purity of compound 28 was assessed at 257 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (Au)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.45</td>
<td>48085</td>
<td>88</td>
</tr>
<tr>
<td>6.81</td>
<td>4453</td>
<td>8</td>
</tr>
<tr>
<td>7.79</td>
<td>2092</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 2-14 illustrates the chromatogram for the calibration curve for 28. The internal standard was tracked at 219 nm while 28 was followed at 257 nm. The calculated slope was used for determination of Michael product 28 formed during the monitored reaction.
Figure 2-14. Calibration Chromatogram and Curve for 28. Calibration curve for compound 28 with concentrations of 5, 10, 15, and 20 µM. Compound 28 was tracked at 257 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve is concentration vs the ratio of the peak area of product 28 (PAP) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.
Figure 2-15 illustrates the chromatograms for the Michael addition reaction with 8. The set of chromatograms at 224 nm show the disappearance of 8 while chromatograms at 257 nm show the appearance of 28 and is highlighted with a box around the gradually growing peak. The middle chromatograms at 219 nm illustrate the internal standard remaining constant and is denoted with an asterisk (*).
Figure 2-15. Change in [8] and [28] During the Michael Addition Reaction. Reactant 8 at 224 nm, internal standard at 219 nm (denoted with an asterisk *), and Michael product 28 at 257 nm, which is highlighted with a box around the growing peak. Chromatograms are represented at time points 0, 1, 24, 48, 72 h. For both the reactant and the product, 97 h is denoted but it is not considered in the calculations and is only used for qualitative information. The data for the Michael product is recorded in concentration due to the presence of a calibration curve.
2.3.4 Compound 9 and 29

Figure 2-16 and Table 2.9 demonstrates the commercially purchased compound 10’s purity and optimal wavelength absorbance. It was found to have a purity of 87% with a retention time of 4.73 min and an optimal wavelength absorbance of 291 nm.

![Figure 2-16. Peak Absorption and Chromatogram for 9. The peak absorption for 9 occurs at 290 nm. This is the wavelength that was used for Michael addition tests with 9.](image)

Table 2.9. Purity Percentage for 9. The purity of compound 9 was assessed at 249 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.73</td>
<td>36784</td>
<td>87</td>
</tr>
<tr>
<td>6.81</td>
<td>5348</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 2-17 depicts the chromatograms generated for the calibration curve for 9. The calculated slope was used to determine the concentration of 9 during the monitored Michael addition reactions.
Figure 2-17. Calibration Chromatogram and Curve for 9. Calibration curve for compound 9 with concentrations of 5, 10, 15, and 20 µM. Compound 9 was tracked at 290 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve is concentration vs the ratio of the peak area of 9 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction test.

\[ y = 12.254x \]
\[ R^2 = 0.9458 \]
The synthesized Michael product 29, shown in Figure 2-18, was found to have a purity of 75% at a retention time of 8.26 min with an optimal wavelength absorbance of 259 nm. Although this is not highly pure, the two impurity peaks correspond to each of the starting materials, namely 9 and thiophenol. Thus, they could be accounted for quantitatively and the peak of interest (8.26 min) could still be used for quantitative calibration of this particular product. Scheme 2-2 depicts the compounds synthetic route.

![Figure 2-18. Peak Absorption and Chromatogram for 29.](image)

The peak absorption for 29 occurs at 259 nm. This is the wavelength that was used for Michael addition tests for 29.

Table 2.10. Purity Percentage for 29. The purity of compound 29 was assessed at 259 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.72</td>
<td>8745</td>
<td>16</td>
</tr>
<tr>
<td>8.26</td>
<td>40367</td>
<td>75</td>
</tr>
<tr>
<td>12.18</td>
<td>4623</td>
<td>9</td>
</tr>
</tbody>
</table>

The chromatograms used to generate the calibration curve is illustrated in Figure 2-19. The slope calculated from the relationship between the known concentration and the ratio of PAP/PAIS was later used to determine the concentration of the product forming over time during the monitored Michael addition reactions.
Figure 2-19. Calibration Chromatogram and Curve for 29. Calibration curve for compound 29 with concentrations of 5, 10, 15, and 20 µM. Compound 29 was tracked at 259 nm while the internal standard, naphthalene, was recorded at 219 nm, denoted with an asterisk (*). The calibration curve is concentration vs the ratio of the peak area of product 29 (PAP) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-20 illustrates the chromatograms for the Michael addition reaction with 29.

The set of chromatograms with 290 nm shows the disappearance of 29 while chromatograms
with 259 nm shows the appearance of 29 and is highlighted with a box around the gradually growing peak. The middle chromatograms measured at 219 nm illustrate the internal standard constant and is denoted with an asterisk (*). It appears that the reaction has two distinctly different rate periods, one during the early time points (0-6 h) and the other after (24-96 h).
Figure 2-20. Change in 9 and 29 During the Michael Addition Reaction. Reactant 9 at 290 nm, internal standard at 219 nm (denoted with an asterisk *), and Michael product 29, at 259 nm which is highlighted with a box around the growing peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 9 is shown as the squares while 29 is shown as circles. The initial and the later equations are shown on the graph. The data for the Michael product is recorded in concentration due to the presence of a calibration curve.
2.3.5 Compound 10

Compound 10 was commercially purchased and was found to have a retention time of 5.33 min with an optimal wavelength absorbance of 257 nm and a purity of 92% which is demonstrated in Figure 2-21.

![Figure 2-21. Peak Absorption and Chromatogram for 10. The peak absorption for 10 occurs at 257 nm. This is the wavelength that was used for Michael addition tests with 10.](image)

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.02</td>
<td>113</td>
<td>1</td>
</tr>
<tr>
<td>4.58</td>
<td>4019</td>
<td>3</td>
</tr>
<tr>
<td>5.33</td>
<td>113691</td>
<td>92</td>
</tr>
<tr>
<td>6.83</td>
<td>4471</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2.11. Purity Percentage for 10. The purity of compound 10 was assessed at 257 nm at a concentration of 20 µM.

Figure 2-22 shows the calibration curve and the chromatograms for compound 10.

The slope generated form these chromatograms were used for the determination of the
concentration of \textbf{10} during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the repetitive nature from previous experiments. Refer to the previous chromatograms for the internal standard measurement.

Figure 2-22. Calibration Chromatogram and Curve for \textbf{10}. Calibration curve for compound \textbf{10} with concentrations of 5, 10, 15, and 20 \(\mu\)M. Compound \textbf{10} was tracked at 257 nm. The internal standard acted the same as in the previous three standards. The calibration curve is concentration vs the ratio of the peak area of \textbf{10} (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-23, shows the chromatograms and graph produced from the Michael addition reaction for compound \textbf{10}. Compound \textbf{10} was monitored at its optimal wavelength, 257 nm. The Michael product was predicted to have a retention time of 6.9
min, which is shown in Figure 2-23, with an optimal wavelength absorption of 251 nm which is shown in Figure 2-25. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-23. This reaction is similar to that of compound 7, as it reacts quickly but not completely. Compound 10 seems to reach an equilibrium after 12 h. The initial equation was generated from 0-6 h while the later equation was generated from 12-96 h. Note, the addition of the TEA had no effect on the product concentration further suggesting that the reaction reached completion.
Figure 2-23. Change in \textbf{10} During the Michael addition reaction. Reactant \textbf{10} at 257 nm. The Michael product peak was predicted to have a retention time of 6.9 min. The Michael product was followed at 251 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, \textbf{10} is shown as the squares and has an initial and later equation. A best fit line is shown on the graph generated by adding the initial and later equations together.

Figure 2-24 depicts the percentage of product forming over time. The immediate formation of product which levels off to a fairly consistent percentage. This correlates with the disappearance of reactant \textbf{10} over time. The \( P_{\text{max}} \) was assumed to be 96 h. The 97 h data is not shown on the graph due to the insignificant change in either the product of reactant.
Figure 2-24. The Percentage of Michael Product Formed from 10 Over Time

This graph was not recalculated to illustrate percentage of product remaining to be formed due to significant negative values which does not display the data in an effective way.

Figure 2-25. Optimal Wavelength of the predicted Michal Addition Product for 10.
The optimal absorbance for the predict Michael product was found to be 251 nm.
Figure 2-26 shows the LCMS SIR scan for the reactant 10 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 10, thiol, and product. There is no evidence that Michael product was formed which could indicate that there is so little formed that detection is not possible. Further investigation of this issue is needed, however, for this thesis it is assumed that Michael product was formed and the predicted peak analyzed above is the Michael product.

Figure 2-26. LCMS SIR for 10. A) The Michael product was detected in the positive mode with a mass of 257.1 g/mol. B) The reactant 10 was detected in the positive mode with a mass of 147.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 10 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 10, Michael addition product and Michael addition donor, thiophenol.
2.3.6 Compound 11

Figure 2-27 and Table 2.12 depicts the optimal wavelength, retention time, and the purity of compound 11 which was synthesized. The synthesis and experimental for this compound can be found in sections 2.4 and 2.6 respectively. The purity for this compound was found to be >99% with a retention time of 8.22 min and an optimal wavelength absorption of 308 nm.

Table 2.12. Purity Percentage for 11. The purity of compound 11 was assessed at 308 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.60</td>
<td>1077</td>
<td>0.5</td>
</tr>
<tr>
<td>8.22</td>
<td>230990</td>
<td>99.5</td>
</tr>
</tbody>
</table>

Figure 2-28 shows the calibration curve and the chromatograms for compound 11. The slope generated form these chromatograms was used for the determination of the
concentration of 11 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

**Figure 2-28. Calibration Chromatogram and Curve for 11.** Calibration curve for compound 11 with concentrations of 5, 10, 15, and 20 µM. Compound 11 was tracked at 308 nm. The internal standard acted the same as in the previous three standards. The calibration curve is concentration vs the ratio of the peak area of 11 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-29, shows the chromatograms and graph produced from the Michael addition reaction for compound 11. Compound 11 was monitored at its optimal
wavelength, 308 nm. The Michael product was predicted to have a retention time of 11.7 min, which is shown in Figure 2-29, with an optimal wavelength absorption of 242 nm which is shown in Figure 2-32. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-29. The initial equation was generated from 0-60 h. Note, the addition of the TEA had no effect of the product concentration further suggesting that the reaction reached completion.
Figure 2-29. Change in [11] During the Michael Addition Reaction. Reactant 11 at 308 nm. The Michael product peak was predicted to have a retention time of 6.9 min. The product was followed at 251 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 11 is shown as the squares and has an initial and later equation.

Figure 2-30 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 11 shown in Figure 2-29.
Figure 2-30. The percentage of Michael Product Formed from 11 Over Time.

Figure 2-31 was generated by subtracting the percentage of the Michael product seen in Figure 2-30 by 100 to achieve the remaining percent of product to be formed. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 11. Both the initial and the later rate values are similar to that of the reactant and product.
Figure 2-31. Percentage of Remaining Michael Product to be Formed. The data was recalculated as 100-percentage of product percent calculated in figure 2-29 in order to determine a rate constant to be compared to the rate constant of the reactant 11.

Figure 2-32. Optimal Wavelength of the predicted Michal Addition Product for 11. The optimal absorbance for the predict Michael product was found to be 242 nm.

Figure 2-33 shows the LCMS SIR scan for the reactant 11 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 11, thiol,
and product. The Michael product can be seen at 2.31 min with an ion mass of 319.1 g/mol while the reactant is at 1.42 min with an ion mass of 209.1 g/mol.

**Figure 2-33. LCMS SIR for 11.** A) The Michael product was detected in the positive mode with a mass of 319.1 g/mol with a retention time of 2.31 min. B) The reactant 11 was detected in the positive mode with a mass of 209.1 g/mol with a retention time of 1.42 min. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 11 and naphthalene. The chromatograms on the right was the 96 h injection which contains reactant 11, Michael addition product and Michael donor, thiophenol.

Figure 2-34 is the LCMS/MS daughter scan for the reactant 11 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 11 (Figure 2-34 C) and the product fragments (Figure 2-34 B). The major daughter ion for the reactant 11 is 103.01 g/mol and 130.89 g/mol while the major ion for the product is 104.83 g/mol. The possible fragments for the reactant 11 and the product can be seen in Table 2-31.
Figure 2-34. Daughter Scan for Time 0 h and 96 h for Michael Addition Reaction for 11. A) Time 0 h daughter scan. At this time point only the reactant 11 and the internal standard, naphthalene, is present. The major fragments found was 103.01 g/mol and 130.89 g/mol. B) Time 96 h at 2.31 min detecting the possible fragments that corresponds to the Michael product parent molecule. The major fragment found was 104.83 g/mol. C) Time 96 h detecting the possible fragments that corresponds to the reactant 11 parent molecule. The major fragments corresponds to panel A. The structure of the possible fragments at this mass is listed in Table 2-31.
2.3.7 Compound 12

Figure 2-35 and Table 2.13 depicts the synthesized compound 12. The optimal wavelength was found to be 316 nm. The retention time was found to be 5.45 min and a purity of > 97%. The synthesis and experimental can be found in sections 2.4 and 2.6 respectively.

![Figure 2-35. Peak Absorption and Chromatogram for 12.](image)
The peak absorption for 12 occurs at 316 nm. This is the wavelength which will be used for Michael addition tests with 12.

**Table 2.13. Purity Percentage for 12.** The purity of compound 12 was detected at 316 nm at a concentration of 20 µM

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.96</td>
<td>1098</td>
<td>1.3</td>
</tr>
<tr>
<td>5.45</td>
<td>85358</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Figure 2-36 show the calibration curve and the chromatograms for compound 12. The slope generated form these chromatograms was used for determination of the concentration of 12 during the Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not show due to the reproducible nature.
from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-36. Calibration Chromatograph and Curve for 12. Calibration curve for compound 12 with concentrations of 5, 10, 15, and 20 µM. Compound 12 was tracked at 316 nm. The internal standard acted the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 12 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-37, shows the chromatograms and graph produced from the Michael addition reaction for compound 12. Compound 12 was monitored at its optimal wavelength, 316 nm. The Michael product was predicted to have a retention time of 8.4 min, which is shown in Figure 2-37, with an optimal wavelength absorption of 219 nm which is shown in Figure 2-40. The internal standard behaved similarly as in previous
reactions, therefore, it is not shown in Figure 2-37. The initial equation was generated from 0-48 h while the later equation was generated from 60-96 h. Note, the addition of the TEA had no effect of the product concentration further suggesting that the reaction reached completion.
Figure 2-37. Change in 12 During the Michael Addition Reaction. Reactant 12 at 316 nm. The Michael product peak was predicted to have a retention time of 8.4 min. The product was followed at 219 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 12 is shown as the squares and has an initial and later equation.

Figure 2-38 depicts the percentage of Michael product forming over time. As seen on the graph in Figure 2-38, the percentage of the product gradually increases over time which correlates with the disappearance of reactant 12 shown in Figure 2-37.

Figure 2-38. The Percentage of Michael Product Formed from 12 Over Time.
Figure 2-39 was generated by subtracting the percentage of the Michael product seen in Figure 2-38 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 12. Both the initial and the later rate values are similar to that of the reactant and product.

**Figure 2-39. Percentage of Remaining of Michael Product to be formed.** The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 12.
Figure 2-40. Optimal Wavelength of the predicted Michal Addition Product for 12. The optimal absorbance for the predict Michael product was found to be 219 nm. Even though 219 nm is not displayed on the graph, the first hump is approximately 219nm.

Figure 2-41 shows the LCMS SIR scan for the reactant 12 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 12, thiol, and product. A peak can be seen at 3.42 min with the mass of 320.1 g/mol which is the mass of the ion of the Michael addition product. The reactant 12 with an ion mass of 210.1 g/mol is seen in both the left and the right panels. This shows that the Michael product was formed and that some of the reactant 12 was still present.
Figure 2-41. LCMS SIR for 12 Michael Addition Reaction. Mobile phase 50/50 ACN/H\textsubscript{2}O 5 min. A) The Michael product was detected in the positive mode with a mass of 320.1 g/mol. B) The reactant 12 was detected in the positive mode with the mass of 210.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with the mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 12 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 12, Michael Addition product and Michael donor, thiophenol.

Figure 2-42 is the LCMS/MS daughter scan for the reactant 12 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 12 (Figure 2-42 C) and the product fragments (Figure 2-42 B). The major daughter ion for the reactant 12 is 182.2 g/mol while the major ion for the product is 107.0 g/mol. The possible fragments for the reactant 12 and the product can be seen in Table 2-31.
Figure 2-42. LCMS/MS Daughter Scan for 12. A) Daughter scan for time 0 h which only contains the starting material 12 and the internal standard naphthalene. The major fragments are 182.16 g/mol, 130.99 g/mol, and 102.97 g/mol. B) Michael addition product formed after 96 h, fragments at retention time 3.42 min with the major fragment being 106.97 g/mol. C) Daughter scan for the starting material 12 remaining in the reaction mixture after 96 h. The fragments match that of panel A.
2.3.8 Compound 13

Figure 2-43 depicts the purity, optimal wavelength and retention time for the synthesized compound 13. The retention time was found to be 5.29 min with an optimal wavelength of 314 nm and a purity of >99%. The synthesis and experimental can be found in section 2.4 and 2.6 respectively.

![Figure 2-43. Peak Absorption and Chromatogram for 13. The peak absorption for 13 occurs at 314 nm. This is the wavelength that was used for Michael addition tests with 13.](image)

Table 2.14. Purity Percentage for 13. The purity of compound 13 was detected at 314 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.48</td>
<td>404</td>
<td>0.4</td>
</tr>
<tr>
<td>5.29</td>
<td>97124</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Figure 2-44 show the calibration curve and the chromatograms for compound 13. The slope generated form these chromatograms were used for the determination of the concentration of 13 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-44. Calibration Chromatograph and Curve for 13. Calibration curve for compound 13 with concentrations of 5, 10, 15, and 20 µM. Compound 13 was tracked at 314 nm. The internal standard identical to the three standards. The calibration curve is concentration vs the ratio of the peak area of 13 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction test.

Figure 2-45, shows the chromatograms and graph produced from the Michael addition reaction for compound 13. Compound 13 was monitored at its optimal wavelength, 314 nm. The Michael product was predicted to have a retention time of 8.1 min, which is shown in Figure 2-45, with an optimal wavelength absorption of 219 nm which is shown in Figure 2-48. The internal standard behaved similarly as in previous
reactions, therefore, it is not shown in Figure 2-45. The initial equation was generated from 0-72 h there is no later equation/rate constant due to few points in the later part of the graph. Note, the addition of the TEA had some effect of the product concentration. The concentration halved between 96 h and 97 h. This indicates that the Michael addition reaction was not complete at 96 h and the addition of TEA pushed the reaction forward.
Figure 2-45. Change in 13 During the Michael Addition Reaction. Reactant 13 at 314 nm. The Michael product peak was predicted to have a retention time of 8.1 min. The product was followed at 219 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 13 is shown as the squares and has an initial equation.

\[
y = 26.79e^{-0.015x} \\
R^2 = 0.897
\]

As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 13 shown in Figure 2-45.
Figure 2-46. The Percentage of Michael Product Formed from 13 Over Time.

Figure 2-47 was generated by subtracting the percentage of the Michael product seen in Figure 2-46 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 13. This data represents the remaining percent of product to be formed.
Figure 2-47. Percent of Remaining Michael Product to be Formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 13.

Figure 2-48 depicts the LCMS SIR scan for the reactant 13 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 13, thiol, and product. A peak can be seen at 3.26 min with the mass of 320.1 g/mol (Figure 2-48 A) which is the mass of the ion of the Michael addition product. The reactant 13 with an ion mass of 210.1 g/mol at 1.35 min (Figure 2-48 B) is seen in both the left and the right panels. This shows that the Michael product was formed and that some of the reactant 13 was still present.
Figure 2-48. LCMS SIR for 13 Michael Addition Reaction. Mobile phase 50/50 ACN/H$_2$O 5 min. A) The Michael product was detected in the positive mode with the mass of 320.1 g/mol. B) The reactant 13 detected in the positive mode with the mass of 210.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with the mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 13 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 13, Michael addition product and Michael donor, thiophenol.

Figure 2-49 depicts the LCMS/MS daughter scan for the reactant 13 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 13 (Figure 2-49 C) and the product fragments (Figure 2-49 B). The major daughter ion for the reactant 13 is 103.0 g/mol and 210.1 g/mol which is the parent molecular weight while the major ion for the product is 106.5 g/mol and 210.3 g/mol. The possible fragments for the reactant 13 and the product can be seen in Table 2.31.
Figure 2-49. LCMS/MS Daughter Scan for 13. A) Daughter scan for time 0 h which only contains the starting material 13 and the internal standard naphthalene. The major fragments are 102.97 g/mol and 131.03 g/mol, B) Michael addition product formed after 96 h at retention time 3.26 min. The major fragments are 210.26 g/mol, 106.48 g/mol, and 182.12 g/mol. C) Daughter scan for the starting material 13 remaining in the reaction mixture after 96 h. The fragments match that of panel A.
2.3.9 Compound 14

Figure 2-50 and Table 2.15 shows the commercially purchased compound 14. The purity was found to be 95% with an optimal wavelength of 286 nm at a retention time of 5.51 min.

Table 2.15. Purity Percentage for 14. The purity of compound 14 was detected at 286 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.51</td>
<td>155277</td>
<td>95</td>
</tr>
<tr>
<td>7.82</td>
<td>7844</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 2-51 show the calibration curve and the chromatograms for compound 14. The slope generated form these chromatograms were used for the determination of the concentration of 14 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the repetitive nature.
from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-51. Calibration Chromatogram and Curve for 14. Calibration curve for compound 14 with concentrations of 5, 10, 15, and 20 µM. Compound 14 was tracked at 286 nm. The internal standard behaved similarly as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 14 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-52, shows the chromatograms and graph produced from the Michael addition reaction for compound 14. Compound 14 was monitored at its optimal wavelength, 286 nm. The Michael product was predicted to have a retention time of 8.6 min, which is shown in Figure 2-52, with an optimal wavelength absorption of 244 nm which is shown in Figure 2-55. The internal standard behaved similarly as in previous
reactions, therefore, it is not shown in Figure 2-52. The initial equation was generated from 0-12 h and a later equation was generated from 24-96 h. Note, the addition of the TEA appears to have no effect on the product concentration which suggest that the reaction reached completion and is not shown on the graph. The concentration of 14 at 97 h was 0.71 µM when compared to 0.38 µM at 96 h. The slight increase is due to the error in removal and processing of the sample.
Figure 2-52. Change in [14] During the Michael Addition Reaction. Reactant 14 at 286 nm. The Michael product peak was predicted to have a retention time of 8.6 min. The product was followed at 244 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 14 is shown as the squares and has an initial and later equation.

\[ y = 10.3606e^{-0.1280x} \]
\[ R^2 = 0.9120 \]

\[ y = 1.9569e^{-0.0233x} \]
\[ R^2 = 0.6087 \]

Figure 2-53 depicts the percentage of Michael product forming from 14 over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 15 shown in Figure 2-52.
Figure 2-53. The Percentage of Michael Product Formed from 14 Over Time.

Figure 2-54 was generated by subtracting the percentage of the Michael product seen in Figure 2-53 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 14. This data gives the percentage of product remaining to be formed.
Figure 2-54. Percent of Remaining Michael Product to be Formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 14. The 12 h data point was excluded from the initial rate calculations to compensate for its atypical reading.

Figure 2-55 shows the LCMS SIR scan for the reactant 14 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 14, thiol, and product. A peak can be seen at 2.14 min with the mass of 320.1 g/mol which is the mass of the ion of the Michael addition product. The reactant 14 with an ion mass of 210.1 g/mol at 1.10 min is seen in both the left and the right panels. This shows that the Michael product was formed and that some of the reactant 14 is still present.
Figure 2-55. LCMS SIR for 14 Michael Addition Reaction. Mobile phase 60/40 ACN/H₂O 5 min. A) The Michael product was detected in the positive mode with a mass of 320.1 g/mol. B) The reactant 14 was detected in the positive mode with a mass of 210.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.1 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 14 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 14, Michael addition product and Michael donor, thiophenol.

Figure 2-56 depicts the LCMS/MS daughter scan for the reactant 14 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 14 (Figure 2-56 C) and the product fragments (Figure 2-56 B). The major daughter ion for the reactant 14 is 182.1 g/mol the major ion for the product is 106.0 g/mol. The possible fragments for the reactant 14 and the product can be seen in Table 2.31.
Figure 2-56. LCMS/MS Daughter Scan for 14. A) Daughter scan for time 0 h which only contains the starting material 14 and the internal standard naphthalene. The major fragment is 182.14 g/mol. B) Michael addition product formed after 96 h with the retention time of 2.14 min. The major fragment is 105.97 g/mol. C) Daughter scan for the starting material 14 remaining in the reaction mixture at retention time 1.1 min after 96 h. The major fragment match that of 0 h.
2.3.10 Compound 15

Figure 2-57 and Table 2.16 show the purity, optimal wavelength absorbance and retention time for the commercially purchased compound 15. The purity was found to be 95% with a retention time of 8.09 min with a maximum absorption of 341 nm.

![Figure 2-57. Peak Absorption and Chromatogram for 15.](image)

The peak absorption for 15 occurs at 341 nm. This is the wavelength that was used for Michael addition tests with 15.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.82</td>
<td>6152</td>
<td>5</td>
</tr>
<tr>
<td>8.09</td>
<td>106629</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 2.16. Purity Percentage for 15. The purity of compound 15 was assessed at 341 nm at a concentration of 20 µM.

Figure 2-58 show the calibration curve and the chromatograms for compound 15.

The slope generated form these chromatograms were used for the determination of the concentration of 15 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.
Figure 2-58. Calibration Chromatograph and Curve for 15. Calibration curve for compound 15 with concentrations of 5, 10, 15, and 20 µM. Compound 15 was tracked at 341 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 15 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-59, shows the chromatograms and graph produced from the Michael addition reaction for compound 15. Compound 15 was monitored at its optimal wavelength, 341 nm. The Michael product was predicted to have a retention time of 8.6 min, which is shown in Figure 2-59, with an optimal wavelength absorption of 237 nm which is shown in Figure 2-62. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-59. This reaction is a slow reaction so there is no initial rate. Note, the addition of the TEA had some effect on the concentration of 15.
which suggest that the reaction nearly reached completion. The concentration of 15 at 97 h was found to be 11.14 µM while at 96 h the concentration was 13.87 µM. This shows that there is some change after adding the base, however, the change was not significant. The small change in concentration indicates that the reaction was near completion.
**Figure 2-59. Change in [15] During the Michael Addition Reaction.** Reactant 15 at 341 nm. The Michael product peak was predicted to have a retention time of 8.6 min. The product was followed at 237 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 15 is shown as the squares. Due to the slow reaction rate, there is only one equation.

\[
y = 19.3519e^{-0.0026x} \\
R^2 = 0.6627
\]

Figure 2-60 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 15 shown in Figure 2-59. The atypical value observed at 72 h likely results from a sampling error made during removal and processing of these particular aliquots by the atypical size of the error bars for just this value.
Figure 2-60. The Percentage of Michael Product Formed from \textbf{15} Over Time.

Figure 2-61 was generated by subtracting the percentage of the Michael product seen in Figure 2-60 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant \textbf{15}. This data gives the percent of remaining Michael product to be formed.
Figure 2-61. Percent of Remaining Michael Product to be Formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 14.

Figure 2-62 shows the LCMS SIR scan for the reactant 15 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 15, thiol, and product. There is no evidence of Michael addition product. This could be because the Michael product is in small concentration and was below the level of detection. The reactant 15 is seen at 1.31 min with an ion mass of 239.1 g/mol (Figure 2-62 B) which is the expected ion mass for 15.
Figure 2-62. LCMS SIR for 15 Michael Addition Reaction. Mobile phase isocratic 70/30 ACN/H₂O 5 min. A) The Michael product was detected in the positive mode with the mass of 349.1 g/mol. B) The reactant 15 was detected in the positive mode with the mass of 239.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with the mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 15 and naphthalene. The chromatograms on the right were the 96 h inject which contains reactant 15, Michael addition product and Michael donor, thiophenol.
2.3.11 Compound 16

Figure 2-63 and Table 2.17 depicts the purity, optimal wavelength absorbance and retention time for the synthesized compound 16. The optimal wavelength was 302 nm with a retention time of 8.30 min and purity of 98%. The synthesis and experimental can be found in sections 2.4 and 2.6 respectively.

![Figure 2-63. Peak Absorption and Chromatogram for 16.](image)
The peak absorption for 16 occurs at 302 nm. This is the wavelength that was used for Michael addition tests with 16.

Table 2.17. Purity Percentage for 16. The purity of compound 16 was assessed at 302 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.46</td>
<td>3903</td>
<td>2</td>
</tr>
<tr>
<td>8.30</td>
<td>237240</td>
<td>98</td>
</tr>
</tbody>
</table>

Figure 2-64 show the calibration curve and the chromatograms for compound 16. The slope generated form these chromatograms were used for the determination of the concentration of 16 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

**Figure 2-64. Calibration Chromatograph and Curve for 16.** Calibration curve for compound 16 with concentrations of 5, 10, 15, and 20 µM. Compound 16 was tracked at 302 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 16 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-65, shows the chromatograms and graph produced from the Michael addition reaction for compound 16. Compound 16 was monitored at its optimal wavelength, 302 nm. The Michael product was predicted to have a retention time of 11.2 min, which is shown in Figure 2-65, with an optimal wavelength absorption of 239 nm which is shown in Figure 2-68. The internal standard behaved similarly as in previous
reactions, therefore, it is not shown in Figure 2-65. This reaction is a slow reaction so there is no initial rate and if there is it is the same rate as the later rate. Note, the addition of the TEA had greater effect during this reaction than the previously listed. This demonstrates the usefulness of the base addition. The reaction was not near complete but with the addition of TEA pushed the reaction further toward completion.
Change in [16] During a Michael Addition Reaction

![Graph showing concentration over time](image)

**Figure 2-65. Change in 16 During the Michael Addition Reaction.** Reactant 16 at 302 nm. The Michael product peak was predicted to have a retention time of 11.2 min. The product was followed at 239 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, 16 is shown as the squares.

Figure 2-66 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 15 shown in Figure 2-65. The 96 h point was assigned the $P_{\text{max}}$ for this reaction which shows the significant increase in the percentage of product formed at 97 h. This corresponds to the data in Figure 2-65 where there is a significant decrease in the reactant 16 at the 97 h time point.
Figure 2-66. The Percentage of Michael Product Formed from 16 Over Time.

Figure 2-67 was generated by subtracting the percentage of the Michael product seen in Figure 2-66 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 16. This data gives the percent of remaining of Michael product to be formed.
Figure 2-67. Percent of Remaining Michael Product to be Formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 16. The initial and the later equations were added together to generate a best fit line which is the solid line.

\[ y = 141.6297e^{-0.0355x} \]
\[ R^2 = 0.6964 \]

Figure 2-68. Optimal wavelength for Michael addition product formed. The optimal wavelength for the predicted Michael Addition Product was found to be 239 nm.

Figure 2-69 shows the LCMS SIR scan for the reactant 16 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 16, thiol,
and product. The Michael product was found with a retention time of 2.10 min with an ion mass of 349.1 g/mol. The reactant 16 was found to have a retention time of 1.4 min with an ion mass of 239.1 g/mol.

Figure 2-69. LCMS SIR for 16 Michael Addition Reaction. Mobile phase isocratic 70/30 ACN/H2O. A) The Michael product was detected in the positive mode with the mass of 349.1 g/mol. B) The reactant 16 was detected in the positive mode with a mass of 239.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 16 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 16, Michael addition product and Michael donor, thiophenol.

Figure 2-70 depicts the LCMS/MS daughter scan for the reactant 16 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 16 (Figure 2-70 C) and the product fragments (Figure 2-70 B). The major daughter ion for the reactant 16 is 133.01 g/mol, 161.04 g/mol, 104.88 g/mol, and 117.95 g/mol while the product fragments have a mass of 104.95 g/mol. The possible fragments for the reactant 16 and the product can be seen in Table 2.31.
Figure 2-70. LCMS/MS Daughter Scan for 16. A) Daughter scan for time 0 h which only contains the starting material 16 and the internal standard naphthalene. The major fragments for 16 are 133.01 g/mol, 161.04 g/mol, 104.88 g/mol, and 117.95 g/mol. B) Michael addition product formed after 96 h. Fragments are at retention time 2.10 min. The major fragment is 104.95 g/mol. C) Daughter scan for the starting material 16 remaining in the reaction mixture after 96 h. The fragmentation corresponds to that of time 0 h.
2.3.12 Compound 17

Figure 2-71 and Table 2.18 depicts the synthesized compound 17. The purity was found to be >99% with an optimal wavelength absorbance of 319 nm and a retention time of 8.05 min. The synthesis and experimental can be found in sections 2.4 and 2.6 respectively.

![Figure 2-71. Peak Absorption and Chromatogram for 17. The peak absorption for 17 occurs at 319 nm. This is the wavelength that was used for Michael addition tests with 17.](image)

**Table 2.18. Purity Percentage for 17.** The purity of compound 17 was assessed at 319 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td>190</td>
<td>0.1</td>
</tr>
<tr>
<td>8.05</td>
<td>276099</td>
<td>99.9</td>
</tr>
</tbody>
</table>

Figure 2-72 show the calibration curve and the chromatograms for compound 17. The slope generated from these chromatograms were used for the determination of the concentration of 17 during the monitored Michael addition reaction. For the remainder of
the chromatograms depicted, the internal standard is not shown due to the reproductive nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

**Figure 2-72. Calibration Chromatograph and Curve for 17.** Calibration curve for compound 17 with concentrations of 5, 10, 15, and 20 µM. Compound 17 was tracked at 319 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 17 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-73, shows the chromatograms and graph produced from the Michael addition reaction for compound 17. Compound 17 was monitored at its optimal wavelength, 319 nm. The Michael product was predicted to have a retention time of 11.3 min, which is shown in Figure 2-73, with an optimal wavelength absorption of 265 nm.
which is shown in Figure 2-76. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-73. The reaction has two rate constants an initial and later. The graph is divided into an initial equation which is from time point 0-36 h and the latter is made up of time points 48-96 h. Note, the addition of the TEA appears to have a no effect on the concentration of 17 which suggests that the reaction had reached or nearly reached completion. The concentration at 97 h was found to be 11.74 ± 21.86 µM while at 96 h the concentration was found to be 1.29 ± 0.20 µM. The increase in concentration at 97 h can be due to error made during the removal and processing of these particular aliquots. This is supported by the large error associated with these sample time point.
Figure 2-73. Change in [17] During the Michael Addition Reaction. Reactant \textbf{17} at 319 nm. The Michael product peak was predicted to have a retention time of 11.3 min. The product was followed at 265 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, \textbf{17} is shown as the squares and has an initial and later equation. From these equations the rate constant can be derived.

Figure 2-74 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant \textbf{16} shown in Figure 2-73.
Figure 2-74. The percentage of Michael product formed over time.

Figure 2-75 was generated by subtracting the percentage of the Michael product seen in Figure 2-74 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 17. This data provides the percent of remaining Michael product to be formed.
**Figure 2-75. Percent of remaining Michael product to be formed.** The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 17. The data from 36 h was excluded from rate calculations due to its atypical reading. This is supported by its large error bars.

**Figure 2-76. Optimal wavelength for Michael addition product formed from 17.** The optimal wavelength for the predicted Michael Addition Product was found to be 265 nm.
Figure 2-77 shows the LCMS SIR scan for the reactant 17 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 17, thiol, and product. The Michael product was found with a retention time of 2.12 min with an ion mass of 349.1 g/mol. The reactant 17 was found to have a retention time of 1.22 min with an ion mass of 239.1 g/mol.

![Figure 2-77. LCMS SIR for 17 Michael Addition Reaction.](image)

The chromatograms on the left was the time 0 h injection which only contains the starting material 17 and naphthalene. The chromatograms on the right was the 96 h injection which contains reactant 17, Michael addition product and Michael donor, thiophenol.

Figure 2-78 depicts the LCMS/MS daughter scan for the reactant 17 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 17 (Figure 2-78 C) and the product fragments (Figure 2-78 B). The major daughter ion for the reactant 17 is 102.92 g/mol, 131.03 g/mol, and 134.94 g/mol while the product fragments have a mass of 135.32 g/mol. The possible fragments for the reactant 17 and the product can be seen in Table 2-31.
Figure 2-78. LCMS/MS Daughter Scan for 17. A) Daughter scan for time 0 h which only contains the starting material 17 and the internal standard naphthalene. The major fragments were 102.92 g/mol and 131.03 g/mol. B) Michael addition product daughter scan after 96 h. The major fragment was 135.32 g/mol. C) Daughter scan for the starting material 17 remaining in the reaction mixture after 96 h. The fragmentation corresponds to that in panel A.
2.3.13 Compound 18

Figure 2-79 and Table 2.19 depicts the synthesized compound 18. The purity was found to be 96% with a retention time of 8.48 min with an optimal wavelength absorption of 309 nm. The synthesis and experimental can be found in sections 2.4 and 2.6 respectively.

Figure 2-79. Peak Absorption and Chromatogram for 18. The peak absorption for 18 occurs at 309 nm. This is the wavelength that was used for Michael addition tests with 18.

Table 2.19. Purity Percentage for 18. The purity of compound 18 was assessed at 309 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.91</td>
<td>892</td>
<td>1</td>
</tr>
<tr>
<td>7.74</td>
<td>2818</td>
<td>3</td>
</tr>
<tr>
<td>8.48</td>
<td>81533</td>
<td>96</td>
</tr>
</tbody>
</table>

Figure 2-80 show the calibration curve and the chromatograms for compound 18. The slope generated form these chromatograms were used for the determination of the concentration of 18 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-80. Calibration Chromatograph and Curve for 18. Calibration curve for compound 18 with concentrations of 5, 10, 15, and 20 µM. Compound 18 was tracked at 309 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 18 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction test.

Figure 2-81, shows the chromatograms and graph produced from the Michael addition reaction for compound 18. Compound 18 was monitored at its optimal wavelength, 309 nm. The Michael product was predicted to have a retention time of 11.7 min, which is shown in Figure 2-81, with an optimal wavelength absorption of 219 nm which is shown in Figure 2-84. The internal standard behaved similarly as in previous
reactions, therefore, it is not shown in Figure 2-81. This reaction is a slow reaction so there is no initial rate and if there is it is the same rate as the later rate. Note, the addition of the TEA had a large effect on the concentration of 18 which suggests that the reaction had not reached completion. This further demonstrate that the addition of base can push the reaction further toward completion.
Figure 2-81. Change in 18 During the Michael Addition Reaction. Reactant 18 at 309 nm. The Michael product peak was predicted to have a retention time of 11.3 min. The product was followed at 219 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. On the graph, $y = 18.8216e^{-0.0094x}$, $R^2 = 0.7930$ is shown the equation which gives the rate constant. The 97 h time point is depicted on the graph due to the significant decrease in the concentration of 18.

Figure 2-82 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 18 shown in Figure 2-81. The $P_{\text{max}}$ was set as the 96 h time point. This shows the significant increase in the product after the addition of the base. This corresponds to the graph in Figure 2-81. The atypical value observed at 72 h is likely the result from a sampling error made during the removal and processing of these aliquots. This interpretation is supported by the atypical size of the error bars for just this value.
Figure 2-82. The percentage of Michael product formed over time.

Figure 2-83 was generated by subtracting the percentage of the Michael product seen in Figure 2-82 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 18. This data gives the percent of remaining product to be formed.
Figure 2-83. Percent of remaining Michael product to be formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 18. The initial and the later equations were added together to generate a best fit line which is the solid line.

\[ y = 102.1276e^{-0.0434x} \]
\[ R^2 = 0.8655 \]

Figure 2-84. Optimal wavelength for Michael addition product formed from 18. The optimal wavelength for the predicted Michael Addition Product was found to be 218 nm, however, 219 nm was used to measure the predicted Michael product.
Figure 2-85 shows the LCMS SIR scan for the reactant 18 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 18, thiol, and product. The Michael product provided a retention time of 2.33 min with a ion mass of 349.1 g/mol. The reactant 17 was found to have a retention time of 1.4 min with an ion mass of 239.1 g/mol.

Figure 2-85. LCMS SIR for 18 Michael Addition Reaction. Mobile phase isocratic 70/30 ACN/H₂O 5 min. A) The Michael product was detected in the positive mode with a mass of 349.1 g/mol. B) The reactant 18 was detected in the positive mode with a mass of 239.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 18 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 18, Michael addition product and Michael donor, thiophenol.

Figure 2-86 is the LCMS/MS daughter scan for the reactant 18 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 18 (Figure 2-86 C) and the product fragments (Figure 2-86 B). The major daughter ion for the reactant 18 is 102.92 g/mol and 131.03 g/mol while the product fragments have a mass
of 135.03 g/mol. The possible fragments for the reactant 18 and the product can be seen in Table 2-31.

Figure 2-86. LCMS/MS Daughter Scan for 18. A) Daughter scan for time 0 h which only contains the starting material 18 and the internal standard naphthalene. The major fragments are 102.92 g/mol and 131.02 g/mol. B) Fragments for Michael product after 96 h at retention time 2.33 min. The major fragment is 135.03 g/mol. C) Daughter scan for the starting material 18 remaining in the reaction mixture after 96 h. The fragments corresponds to that in panel A.
2.3.14 Compound 19

Figure 2-87 and Table 2.20 depicts the purity, optimal wavelength absorbance and retention time of the synthesized compound 19. It was found to have a purity of > 99% with an optimal wavelength absorption of 400 nm at a retention time of 6.62 min. The synthesis and experimental can be found in sections 2.4 and 2.6 respectively.

Table 2.20. Purity Percentage for 19. The purity of compound 19 was detected at 400 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.62</td>
<td>266451</td>
<td>99.5</td>
</tr>
<tr>
<td>16.37</td>
<td>1289</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 2-88 show the calibration curve and the chromatograms for compound 19. The slope generated form these chromatograms were used for the determination of the concentration of 19 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-88. Calibration Chromatograph and Curve for 19. Calibration curve for compound 19 with concentrations of 5, 10, 15, and 20 µM. Compound 19 was tracked at 400 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 19 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction test.

Figure 2-89, shows the chromatograms and graph produced from the Michael addition reaction for compound 19. Compound 19 was monitored at its optimal wavelength, 400 nm. This compound 19 does not undergo a Michael addition reaction. The
graph in Figure 2-89 shows a fairly level trend line indicating that there is little Michael addition occurring. Even after the addition of TEA, there was no change in reactivity.

Figure 2-89. Change in 19 during the Michael addition reaction. Reactant 19 at 400 nm. There is no change in the [19] during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition.
Since there was no evidence that a Michael product was forming, LCMS study was conducted to confirm that there was no product present. Figure 2-90 show the LCMS data for 19 before the reaction occurs and after the reaction had been ran for 96 h. The reactant 19 can be seen at 2.36 min with an ion mass of 292.1 g/mol and there is no ion with a mass of 402.1 g/mol (Michael product mass). This was done with an isocratic method of 50/50 ACN/H₂O for 5 min (Figure 2-90 A). To ensure that the product was not coming out later the method was changed to 60/40 ACN/H₂O for 5 min (Figure 2-90 B) moving the reactant 19 to 0.7 min and there is still no evidence that Michael product was formed.
Figure 2-90. LCMS SIR for 19 Michael Addition Reaction. A) Mobile phase 50/50 ACN/H₂O, 5min. B) Mobile phase 60/40 ACN/H₂O, 5min. A-1) The Michael product was detected in the positive mode with a mass of 402.1 g/mol. A-2) The reactant 19 was detected in the positive mode with a mass of 292.1 g/mol. A-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. B-1) The Michael product was detected in the positive mode with a mass of 402.1 g/mol. B-2) The reactant 19 was detected in the positive mode with a mass of 292.1 g/mol. B-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 19 and naphthalene. The chromatograms on the right was the 96 h inject which contains reactant 19, Michael addition product and the Michael donor, thiophenol.
2.3.15 Compound 20

Figure 2-91 and Table 2.21 shows the purity, optimal wavelength absorption and retention time of compound 20 which was previously synthesized. Compound 20 was found to have a purity of 99% at an optimal wavelength of 416 nm and a retention time of 4.91 min.

Figure 2-91. Peak Absorption and Chromatogram for 20. The peak absorption for 20 occurs at 416 nm. This is the wavelength that was used for Michael addition tests with 20.

Table 2.21. Purity Percentage 20. The purity of compound 20 was detected at 416 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.91</td>
<td>111285</td>
<td>99</td>
</tr>
<tr>
<td>16.24</td>
<td>824</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2-92 show the calibration curve and the chromatograms for compound 20. The slope generated from these chromatograms was used for the determination of the concentration of 20 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-92. Calibration Chromatogram and Curve for 20. Calibration curve for compound 20 with concentrations of 5, 10, 15, and 20 µM. Compound 20 was tracked at 400 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 20 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-93, shows the chromatograms and graph produced from the Michael addition reaction for compound 20. Compound 20 was monitored at its optimal wavelength, 416 nm. This compound 20 does not participate in Michael addition reaction. The graph in Figure 2-93 shows a fairly level trend line indicating that there is little Michael addition occurring. Even after the addition of TEA, there was no change in reactivity.
Figure 2-93. Change in 20 During the Michael Addition Reaction. Reactant 20 at 416 nm. There is no change in 20 during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition.

Since this compound is similar in structure to compound 20, there was no need to further study this compound and reaction with the LCMS.
2.3.16 Compound 21

Figure 2-94 and Table 2.22 shows the purity, optimal wavelength absorption and retention time of compound 21 which was synthesized previously. Compound 21 was found to have a purity of 99% at an optimal wavelength of 406 nm and a retention time of 4.55 min.

Figure 2-94. Peak Absorption and Chromatogram for 21. The peak absorption for 21 occurs at 406 nm. This is the wavelength which will be used for Michael addition tests with 21.

Table 2.22. Purity Percentage 21. The purity of compound 21 was detected at 406 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.31</td>
<td>862</td>
<td>1</td>
</tr>
<tr>
<td>4.55</td>
<td>253186</td>
<td>99</td>
</tr>
</tbody>
</table>

Figure 2-95 show the calibration curve and the chromatograms for compound 21. The slope generated from these chromatograms was used for the determination of the concentration of 21 during the monitored Michael addition reaction. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

![Calibration Chromatograph and Curve for 21](image)

**Figure 2-95. Calibration Chromatograph and Curve for 21.** Calibration curve for compound 21 with concentrations of 5, 10, 15, and 20 µM. Compound 21 was tracked at 400 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 21 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-96, shows the chromatograms and graph produced from the Michael addition reaction for compound 21. Compound 21 was monitored at its optimal wavelength, 406 nm. This compound 21 does not participate in Michael addition reaction. The graph in Figure 2-96 shows a fairly level trend line indicating that there is little Michael
addition occurring. Even after the addition of TEA, there was no change in reactivity. Since this compound is similar in structure as the previous two, no LCMS study was conducted.

**Figure 2-96. Change in 21 During the Michael Addition Reaction.** Reactant 21 at 406 nm. There is no change in 21 during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition.
2.3.17 Compound 1

Figure 2-97 and Table 2.23 depicts the purity, optimal wavelength absorption and retention time for synthesized compound 1. The purity was found to be 98% with an optimal wavelength absorption of 420 nm and a retention time of 4.83 min. The synthesis and experimental can be found in section 2.4 and 2.6 respectively.

Table 2.23. Purity Percentage 1. The purity of compound 1 was detected at 420 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.60</td>
<td>1841</td>
<td>1</td>
</tr>
<tr>
<td>4.83</td>
<td>218592</td>
<td>98</td>
</tr>
<tr>
<td>8.10</td>
<td>1485</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2-98 illustrates the calibration curve and the chromatograms for compound 1. The slope generated from these chromatograms was used for the determination of the concentration of 1 during the monitored Michael addition reaction. For the remainder of
the chromatograms depicted, the internal standard is not shown due to the reproducible nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-98. Calibration Chromatograph and Curve for 1. Calibration curve for compound 1 with concentrations of 5, 10, 15, and 20 µM. Compound 1 was tracked at 420 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 1 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-99, shows the chromatograms and graph produced from the Michael addition reaction for compound 1. Compound 1 was monitored at its optimal wavelength, 420 nm. This compound 1 does not participate in Michael addition reaction. The graph in
Figure 2.99 shows a fairly level trend line indicating that there is little Michael addition occurring. Even after the addition of TEA, there was no change in reactivity.

**Figure 2.99. Change in $\mathbf{1}$ During the Michael Addition Reaction.** Reactant $\mathbf{1}$ at 406 nm. There is no change in $\mathbf{1}$ during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant suggesting no Michael addition.
Since there was no evidence that a Michael product was forming, an LCMS study was conducted to confirm that there was no product present. Figure 2-100 shows the LCMS data for 1 before the reaction occurs and after the reaction had been run for 96 h. The reactant 1 can be seen at 0.98 min with an ion mass of 293.1 g/mol and there is no ion with a mass of 403.1 g/mol (Michael product mass). This was done with an isocratic method of 50/50 ACN/H$_2$O for 10 min. To ensure that the product was not coming out later, the method was changed to 80/20 ACN/H$_2$O for 10 min moving the reactant 1 to 0.7 min. There is still no evidence that a Michael product was being formed.
Figure 2-100. LCMS SIR for 1 Michael Addition Reaction. A) Mobile phase 50/50 ACN/H2O, 10min. B) Mobile phase 80/20 ACN/H2O, 10min. A-1) The Michael product was detected in the positive mode with a mass of 403.1 g/mol. A-2) The reactant 1 was detected in the positive mode with a mass of 293.1 g/mol. A-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. B-1) The Michael product was detected in the positive mode with a mass of 403.1 g/mol. B-2) The reactant 1 was detected in the positive mode with a mass of 293.1 g/mol. B-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left were the time 0 h injection which only contains the starting material 1 and naphthalene. The chromatograms on the right were the 96 h inject which contains reactant 1, Michael addition product and Michael donor, thiophenol.
2.3.18 Compound 2

Figure 2-101 and Table 2.24 shows the purity, optimal wavelength and the retention time for compound 2, synthesized previously. The purity was found to be 96% at the optimal wavelength of 222 nm at a retention time of 6.42 min.

![Chromatogram and Peak Absorption](image)

Figure 2-101. Peak Absorption and Chromatogram for 2. The peak absorption for 2 occurs at 222 nm. This is the wavelength that was used for Michael addition tests with 2.

Table 2.24. Purity Percentage of 2. The purity of compound 2 was assessed at 222 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.54</td>
<td>2763</td>
<td>4</td>
</tr>
<tr>
<td>6.42</td>
<td>67423</td>
<td>96</td>
</tr>
</tbody>
</table>

Figure 2-102 show the calibration curve and the chromatograms for compound 2. The slope generated from these chromatograms was used for the determination of the concentration of 2 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-102. Calibration Chromatogram and Curve for 2. Calibration curve for compound 2 with concentrations of 5, 10, 15, and 20 µM. Compound 2 was tracked at 222 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 2 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-103, shows the chromatograms and graph produced from the Michael addition reactions for compound 2. Compound 2 was monitored at its optimal wavelength, 222 nm. The Michael product was predicted to have a retention time of 8.5 min, which is shown in Figure 2-103, with an optimal wavelength absorption of 215 nm which is shown
in Figure 2-106. The internal standard behaved similarly as in previous experiments. Therefore, it is not shown in Figure 2-103. This reaction was slow, however an initial rate generated from time 0-48 h and a later rate generated from 60-96 h. Also note that the addition of the TEA caused the concentration of 2 to increase rather than decrease. Once the base was added to the reaction mixture, there was a dramatic color change from yellow to red. This indicates that the base affected 2 in such a way that it increased its UV response.
Figure 2-103. Change in [2] During the Michael addition reaction. Reactant 2 at 222 nm. There is no change in 2 during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. Depicted on the graph, 2 is shown in the squares with two equations, an initial and later. From these rate constants can be determined.

Figure 2-104 depicts the percentage of Michael product forming over time. As can be seen on the graph, the percentage of the product gradually increases over time which correlates with the disappearance of reactant 2 shown in Figure 2-103. The atypical value observed at 48 h likely results from a sampling error made during removal and processing of these particular aliquots. This interpretation is supported by the atypical size of the error bars for just this value. This phenomenon is seen in both Figure 2-104 and Figure 2-105.
Figure 2-104. The percentage of Michael product formed over time.

Figure 2-105 was generated by subtracting the percentage of the Michael product seen in Figure 2-104 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 2. This data gives the percent of remaining Michael product to be formed.
Figure 2-105. Percent of remaining Michael product to be formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 2. The data collected from 48 h was excluded from rate calculations due to its atypical value. This is supported by the large error bars.

Figure 2-106. Optimal wavelength for Michael addition product formed from 2. The optimal wavelength for the predicted Michael Addition Product was found to be 213 nm.
Figure 2-107 shows the LCMS SIR scan for the reactant 2 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 2, thiol, and product. The Michael product was found with a retention time of 3.72 min with an ion mass of 457.1 g/mol. The reactant 2 was found to have a retention time of 1.6 min with an ion mass of 347.1 g/mol.

![Figure 2-107. LCMS SIR for 2 Michael Addition Reaction.](image)

A) The Michael product was detected in the positive mode with a mass of 457.1 g/mol. B) The reactant 2 detected in the positive mode with a mass of 347.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with the mass of 110.1 g/mol. The chromatograms on the left were the time 0 h injection which only contains the starting material 2 and naphthalene. The chromatograms on the right were the 96 h inject which contains reactant 2, Michael addition product and Michael donor, thiophenol.

Figure 2-108 is the LCMS/MS daughter scan for the reactant 2 at time 0 h which is seen in the left panel and 96 h is seen in the right panel with daughter ions for the reactant 2 (Figure 2-108 C) and the product fragments (Figure 2-108 B). The major daughter ion for the reactant 2 is 347.0 g/mol, 263.0 g/mol, 105.89 g/mol and 277.93 g/mol while the
product fragments have a mass of 121.97 g/mol. The possible fragments for the reactant 2 and the product can be seen in Table 2.31.

**Figure 2-108. LCMS/MS Daughter Scan for 2.** A) Daughter scan for time 0 h which only contains the starting material 2 and the internal standard naphthalene. The major fragments are 347.00 g/mol, 203.1 g/mol, 106.89 g/mol, and 277.93 g/mol. B) Michael addition product formed after 96 h at the retention time of 3.72 min. The major fragments 121.97 g/mol. C) Daughter scan for the starting material 2 remaining in the reaction mixture after 96 h. The fragments match that of time 0 h.
2.3.19 Compound 22

Figure 2-109 and Table 2.25 depicts the purity, optimal wavelength absorption and the retention time for compound 22 synthesized previously. Compound 22 was found to have a purity of 96% at an optimal wavelength absorption of 291 nm with a retention time of 5.42 min.

![Image of peak absorption and chromatogram for 22](image)

**Figure 2-109. Peak Absorption and Chromatogram for 22.** The peak absorption for 22 occurs at 291 nm. This is the wavelength that was used for Michael addition tests with 22.

**Table 2.25. Purity Percentage for 22.** The purity of compound 22 was assessed at 291 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.42</td>
<td>24457</td>
<td>96</td>
</tr>
<tr>
<td>6.87</td>
<td>1126</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 2-110 show the calibration curve and the chromatograms for compound 22. The slope generated from these chromatograms was used for the determination of the
concentration of 22 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-110. Calibration Chromatogram and Curve for 22. Calibration curve for compound 22 with concentrations of 5, 10, 15, and 20 µM. Compound 22 was tracked at 291 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 22 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-111, shows the chromatograms and graph produced from the Michael addition reaction for compound 22. Compound 22 was monitored at its optimal
wavelength, 291 nm. This compound \textbf{22} does not participate in Michael addition reaction. The graph in Figure 2-111 shows a fairly level trend line indicating that there is little Michael addition occurring. Even after the addition of TEA, there was no change in reactivity.

\textbf{Figure 2-111. Change in \textbf{22} During the Michael Addition Reaction.} Reactant \textbf{22} at 291 nm. There is no change in \textbf{22} during the course of the Michael addition reaction.
Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition.

Since there was no evidence that a Michael product was forming, LCMS study was conducted to confirm that there was no product present. Figure 2-112 shows the LCMS data for 22 before the reaction occurs and after the reaction had been ran for 96 h. The reactant 22 can be seen at 1.2 min with an ion mass of 337.1 g/mol and there is no ion with a mass of 447.1g/mol (Michael product mass). This was done with an isocratic method of 50/50 ACN/H$_2$O for 10 min (Figure 2-112 A). To ensure that the product was not coming out later the method was changed to 80/20 ACN/H$_2$O for 10 min (Figure 2-112 B) moving the reactant 22 to 0.8 min and there is still no evidence that Michael product was formed.
Figure 2-112. LCMS SIR for 22 Michael Addition Reaction. A) Mobile phase 50/50 ACN/H₂O, 10 min. B) Mobile phase 80/20 ACN/H₂O, 10 min. A-1) The Michael product was detected in the positive mode with the mass of 447.1 g/mol. A-2) The reactant 22 was detected in the positive mode with the mass of 337.1 g/mol. A-3) The Michael donor, thiophenol, was detected in the positive mode with the mass of g/mol. B-1) The Michael product was detected in the positive mode with the mass of 447.1 g/mol. B-2) The reactant 22 was detected in the positive mode with the mass of 337.1 g/mol. B-3) The Michael donor, thiophenol, was detected in the positive mode with the mass of g/mol. The chromatograms on the left were the time 0 h injection which only contains the starting material 22 and naphthalene. The chromatograms on the right were the 96 h inject which contains the reactant 22, Michael addition product and Michael donor, thiophenol.
2.3.20 Compound 23

Figure 2-113 and Table 2.26 depicts the purity, optimal wavelength absorption and retention time for compound 23 which was synthesized previously. It was found that 23 was found to have a purity of 98% at an optimal wavelength of 410 nm at a retention time of 4.63 min.

![Figure 2-113. Peak Absorption and Chromatograph for 23.](image)

The peak absorption for 23 occurs at 410 nm. This is the wavelength that was used for Michael addition tests with 23.

Table 2.26. Purity Percentage for 23. The purity of compound 23 was assessed at 410 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.89</td>
<td>1058</td>
<td>2</td>
</tr>
<tr>
<td>4.63</td>
<td>55076</td>
<td>98</td>
</tr>
</tbody>
</table>

![Figure 2-114. Calibration Curve and Chromatograms for 23.](image)

Figure 2-114 show the calibration curve and the chromatograms for compound 23. The slope generated from these chromatograms were used for the determination of the concentration of 23 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible nature from the previous experiments. Refer to the previous chromatograms for the internal standard measurements.
Figure 2-114. Calibration Chromatogram and Curve for 23. Calibration curve for compound 23 with concentrations of 5, 10, 15, and 20 µM. Compound 23 was tracked at 410 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 23 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-115, shows the chromatograms and graph produced from the Michael addition reaction for compound 23. Compound 23 was monitored at its optimal wavelength, 410 nm. This compound 23 does not participate in Michael addition reaction. The graph in Figure 2-113 shows a fairly level trend line indicating that there is little Michael addition occurring. After the addition of TEA, there was no change in reactivity. The concentration of 23 at 97 h 73.11 ± 28.65 µM while the concentration at 96 h was found to be 68.57 ± 27.80 µM. Since this compound is similar in structure as the previous two, no LCMS study was conducted.
Figure 2-115. Change in 23 During the Michael Addition Reaction. Reactant 23 at 410 nm. There is no change in 23 during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition occurred.
2.3.21 Compound 24

Figure 2-116 and Table 2.27 depicts the purity, optimal wavelength and retention time for compound 24 which was synthesized previously. Compound 24 was found to have a purity of 99% at the optimal wavelength of 229 nm with a retention time of 4.71 min.

Figure 2-116. Peak Absorption and Chromatogram for 24. The peak absorption for 24 occurs at 229 nm. This is the wavelength that was used for Michael addition tests with 24.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.96</td>
<td>1353</td>
<td>1</td>
</tr>
<tr>
<td>4.71</td>
<td>141141</td>
<td>99</td>
</tr>
</tbody>
</table>

Table 2.27. Purity Percentage for 24. The purity of compound 24 was assessed at 229 nm at a concentration of 20 µM.

Figure 2-117 show the calibration curve and the chromatograms for compound 24. The slope generated from these chromatograms was used for the determination of the concentration of 24 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.
Figure 2-117. Calibration Chromatograph and Curve for 24. Calibration curve for compound 24 with concentrations of 5, 10, 15, and 20 µM. Compound 24 was tracked at 229 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 24 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-118, shows the chromatograms and graph produced from the Michael addition reaction for compound 24. Compound 24 was monitored at its optimal wavelength, 229 nm. This compound 24 did not participate in Michael addition reaction. The graph in Figure 2-118 shows a fairly level trend line indicating that there is little Michael addition occurring. Even after the addition of TEA, there was no change in reactivity. The variation in the concentration of 24 over time can be explained by this reaction only being an n = 1.
Figure 2-118. Change in 24 during the Michael addition reaction. Reactant 24 at 229 nm. There is no change in 24 during the course of the Michael addition reaction. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows that the slope of the trend line remains fairly constant showing no Michael addition.

Since there was no evidence that a Michael product was forming, LCMS study was conducted to confirm that there was no product present. Figure 2-119 show the LCMS data for 24 before the reaction occurs, 0 h, and after the reaction had been ran for 96 h. The
reactant \textbf{24} can be seen at 1 min with an ion mass of 293.1 g/mol and there is no ion with a mass of 403.1 g/mol (Michael product mass). This was done with an isocratic method of 50/50 ACN/H$_2$O for 10 min (Figure 2-119 A). To ensure that the product was not coming out later the method was changed to 80/20 ACN/H$_2$O for 10 min (Figure 2-119 B) moving the reactant \textbf{24} to 0.7 min and there is still no evidence that Michael product was formed.
Figure 2-119. LCMS SIR for 24 Michael Addition Reaction. A) Mobile phase 50/50 ACN/H$_2$O, 10min. B) Mobile phase 80/20 ACN/H$_2$O, 10min. A-1) The Michael product was detected in the positive mode with a mass of 403.1 g/mol. A-2) The reactant 24 was detected in the positive mode with a mass of 293.1 g/mol. A-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. B-1) The Michael product was detected in the positive mode with a mass of 403.1 g/mol. B-2) The reactant 24 was detected in the positive mode with a mass of 293.1 g/mol. B-3) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left were the time 0 h injection which only contains the starting material 24 and naphthalene. The chromatograms on the right were the 96 h inject which should contain the reactant 24, Michael addition product and Michael donor, thiophenol.
2.3.22 Compound 25

Figure 2-120 and Table 2.28 depicts the purity, optimal wavelength and retention time for commercially purchased compound 25. Compound 25 was found to have a purity of 97% at the optimal wavelength of 216 nm with a retention time of 2.97 min.

![Figure 2-120. Peak Absorption and Chromatogram for 25.](image)

The peak absorption for 25 occurs at 216 nm. This is the wavelength that was used for Michael addition tests with 25.

Table 2.28. Purity Percentage for 25. The purity of compound 25 was assessed at 216 nm at a concentration of 20 μM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.77</td>
<td>4158</td>
<td>3</td>
</tr>
<tr>
<td>2.97</td>
<td>139106</td>
<td>97</td>
</tr>
</tbody>
</table>

Figure 2-121 show the calibration curve and the chromatograms for compound 25. The slope generated form these chromatograms were used for the determination of the concentration of 25 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

Figure 2-121. Calibration Chromatogram and Curve for 25. Calibration curve for compound 25 with concentrations of 5, 10, 15, and 20 µM. Compound 25 was tracked at 216 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 25 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-122, shows the chromatograms and graph produced from the Michael addition reaction for compound 25. Compound 25 was monitored at its optimal wavelength, 216 nm. The Michael product was predicted to have a retention time of 4.0 min, which is shown in Figure 2-122, with an optimal wavelength absorption of 257 nm.
which is shown in Figure 2-125. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-122. There is an initial rate which is calculated from time points 0-24 h and then the later rate is calculated from time points 36-96 h. Note, the addition of the TEA seems to have no effect of the product concentration which suggest that the reaction reached its completion. The concentration of 25 at 97 h was found to be 5.51 ± 1.78 µM while the concentration at 96 h was 5.98 ± 1.77 µM.
**Figure 2-122. Change in [25] During the Michael Addition Reaction.** Reactant 25 at 216 nm. The Michael product peak was predicted to have a retention time of 4.0 min. The product was followed at 257 nm and highlighted with a box around the predicted product peak. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows an initial and latter reaction rate illustrated by two equations.

Figure 2-123 depicts the percentage of Michael product forming over time. As can be seen on the graph the percentage of the product gradually increases over time which correlates with the disappearance of reactant 25 shown in Figure 2-122.
Figure 2-123. The percentage of Michael product formed over time.

Figure 2-124 was generated by subtracting the percentage of the Michael product seen in Figure 2-123 by 100. This causes the graph to become an exponential graph which can help compare the rate constants of the predicted product to that of the reactant 25. This data gives the percent of remaining Michael product to be formed.
Figure 2-124. Percent of remaining Michael product to be formed. The data was recalculated as 100-percentage of product in order to determine a rate constant to be compared to the rate constant of the reactant 25. The initial and the later equations were added together to generate a best fit line which is the solid line.

Figure 2-125. Optimal wavelength for Michael addition product formed from 25. The optimal wavelength for the predicted Michael Addition Product was found to be 257 nm.
Figure 2-126 shows the LCMS SIR scan for the reactant 25 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 25, thiol, and product. The Michael product was found with a retention time of 0.98 min with an ion mass of 148.1 g/mol. The reactant 25 was found to have a retention time of 1.28 min with an ion mass of 258.1 g/mol. However, when a daughter ion scan was preformed there was no significant ion fragments for the possible Michael product parent ion. This could suggest a low concentration of the Michael product formed. It is important to note that this reaction was cumbersome and possessed a tedious purification. It was obvious that there are side reactions or possible degradation occurring due to the appearance of multiple peaks over time. It made it challenging to identifying the Michael addition product. For the purpose of this thesis, it is assumed at the assigned peak in Figure 2-123 is the Michael product and that the side reactions or degradation is irrelevant.
Figure 2-126. LCMS SIR for 25 Michael Addition Reaction. Mobile phase 40/60 ACN/H₂O 5 min. A) The Michael product was detected in the positive mode with a mass of 258.1 g/mol. B) The reactant 25 was detected in the positive mode with a mass of 148.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with a mass of 110.0 g/mol. The chromatograms on the left were the time 0 h injection which only contains the starting material 25 and naphthalene. The chromatograms on the right were the 96 h inject which contains reactant 25, Michael addition product and Michael donor, thiophenol.
2.3.23 Compound 26

Figure 2-127 and Table 2.29 depicts the purity, optimal wavelength absorption and the retention time for synthesized compound 26. The purity was found to be 99% at an optimal wavelength of 292 nm at a retention time of 5.88 min. The synthesis and experimental can be found in section 2.4 and 2.6 respectively.

![Image of chromatogram and peak absorption](image.png)

**Figure 2-127. Peak Absorption and Chromatograph for 26.** The peak absorption for 26 occurs at 292 nm. This is the wavelength that was used for Michael addition tests with 26.

**Table 2.29. Purity Percentage for 26.** The purity of compound 26 was detected at 292 nm at a concentration of 20 µM.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area (AU)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.88</td>
<td>127495</td>
<td>99</td>
</tr>
<tr>
<td>12.15</td>
<td>860</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2-128 show the calibration curve and the chromatograms for compound 26. The slope generated from these chromatograms was used for the determination of the concentration of 26 during the monitored Michael addition reactions. For the remainder of the chromatograms depicted, the internal standard is not shown due to the reproducible
nature from previous experiments. Refer to the previous chromatograms for the internal standard measurements.

**Figure 2-128. Calibration Chromatogram and Curve for 26.** Calibration curve for compound 26 with concentrations of 5, 10, 15, and 20 µM. Compound 26 was tracked at 292 nm. The internal standard behaved the same as in the three standards. The calibration curve is concentration vs the ratio of the peak area of 26 (PAR) over the peak area of the internal standard (PAIS). The slope of the resulting calibration curve was used for calculations during the Michael addition reaction tests.

Figure 2-129, shows the chromatograms and graph produced from the Michael addition reaction for compound 26. Compound 26 was monitored at its optimal wavelength, 292 nm. The Michael product could not be predicted due to multiple peak forming over time. Therefore, only the reactant 26 was monitored over time. The internal standard behaved similarly as in previous reactions, therefore, it is not shown in Figure 2-129.
129. There is an initial rate which is calculated from time points 0-24 h and then the later rate is calculated from time points 36-96 h. Note, the addition of the TEA appears to have no effect of the product concentration which suggest that the reaction reached its completion. The concentration of 26 was found to be 2.30 ± 0.36 µM at 97 h while at 96 h the concentration was 2.85 ± 0.50 µM.
Figure 2-129. Change in 26 During the Michael Addition Reaction. Reactant 26 at 292 nm. The Michael product peak was unable to be predicted and therefore was not followed. Chromatograms are represented at time points 0, 1, 24, 48, 72, and 96 h. The graph shows an initial and later reaction rate illustrated by two equations.

Figure 2-130 shows the LCMS SIR scan for the reactant 26 and the Michael product. The panel on the left which was the reaction time 96 h should contain reactant 26.
thiol, and product. The Michael product was found with a retention time of 1.29 min with an ion mass of 224.1 g/mol. The reactant 26 was found to have a retention time of 2.24 min with an ion mass of 334.1 g/mol. However, when a daughter ion scan was preformed there was no significant ion fragments for the possible Michael product parent ion. This could suggest a low concentration of the Michael product formed. It is important to note that this reaction was not as straight forward as the previous reactions. It was obvious that there are side reactions or possible degradation occurring due to the appearance of multiple peaks over time. It made it challenging in identifying the possible Michael addition product. For the purpose of this thesis, it is assumed that Michael product is forming even if at low concentrations. Further investigation of this compound will be performed to identify the extent of completion.

Figure 2-130. LCMS SIR for 26 Michael Addition Reaction. Mobile phase 60/40 ACN/H2O 5 min. A) The Michael product was detected in the positive mode with the mass of 334.1 g/mol. B) The reactant 26 was detected in the positive mode with the mass of 224.1 g/mol. C) The Michael donor, thiophenol, was detected in the positive mode with the mass of 110.0 g/mol. The chromatograms on the left was the time 0 h injection which only contains the starting material 26 and naphthalene. The chromatograms on the right was the 96 h inject which should contain reactant 26, Michael addition product and Michael donor, thiophenol.
2.3.24 Results Summary and Discussion

There were complications with mass balance during the course of the Michael addition reaction study. The mass balances during the standard reactions were off by about 20-30%. Some studies were conducted to determine explanations for this issue. One study examined the effect of the reactant on the product’s UV response. The reactant was treated with 75, 50, and 25% of product to determine if the UV response, in either the product or the reactant, would altered at various concentrations. There was little effect on either the reactant or the product’s peak area at any concentration. A second study separately examined decomposition of either the pure reactant or the pure product under the reaction conditions (DCM/MeOH no base) for 96 h. Results again showed little change in the peak area of either the reactant or product. Other explanations could include the possibility that some of the mass remained as the chemical intermediate (refer to Scheme 1-1 in chapter 1) which may collapse to other chemical species when processing samples for analyses. Alternatively, degradation of product or reactant could be occurring, but only when in the presence of each other or in presence of the thiol.

Table 2.30 lists the α,β-unsaturated ketones with various groups located on either side of the α,β-unsaturated ketone, as well as the respective rate constants (k) calculated from the Michael addition reaction graphs previous listed. This reaction is a first order rate reaction having an equation of \( y = ae^{bx} \), where \( k = -b \), the equations from the initial portion (when applicable) to the reactant graph was used. For example, compound 26 depicted in the previous section 2.2.23, the equation generated from the Michael addition reaction graph seen in Figure 2-127 has an initial equation of \( y = 14.7848e^{(-0.0589x)} \). Therefore, \( k = -b \) would be \( k = -(0.0589) \) which gives \( k = 0.0589 \) per h. The rate constant
where then compared to each other and divided into four categories: high reactivity with a rate constant of > 0.1 per h, moderate reactivity with the rate constant of 0.01-0.09 per h, low reactivity with a rate constant of 0.001-0.009 per h and non-reactive with a rate constant of < 9x10^-4 per h. All compounds fall into the categories efficiently with exception of three: 21, 1, and 24 all of which as denoted in Table 2.30. These compounds were found to have a k value that would classify it as low reactive compounds however during the study there was no evidence of a Michael product forming over time. Therefore these compounds was categorized as non-reactive.
Table 2.30. Michael Addition Reactivity. * compounds which placement needs to be discussed.

![Michael Addition Reactivity Diagram]

<table>
<thead>
<tr>
<th>Compound #</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>k (per h)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Reactivity (k &gt;0.1 per h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>H-</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>6.315</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ph-</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>0.1501</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>Ph-</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Ph-</td>
<td>Ph-</td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td><strong>Moderate Reactivity (k = 0.01-0.09 per h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>0.04199</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Ph-</td>
<td></td>
<td>0.03338</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Ph-</td>
<td></td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ph-</td>
<td></td>
<td>0.0421</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Ph-</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>0.0186</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Ph-</td>
<td></td>
<td>0.0589</td>
<td></td>
</tr>
<tr>
<td><strong>Low Reactivity (k = 0.001-0.009 per h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>0.0026</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>0.0068</td>
<td></td>
</tr>
<tr>
<td>( \text{Ph} )</td>
<td></td>
<td></td>
<td>( 0.0094 )</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Non-reactive ( (k &lt; 9 \times 10^{-4} \text{ per h}) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 18 )</td>
<td>Ph-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 19 )</td>
<td>Ph-</td>
<td></td>
<td>9 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>7 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>21*</td>
<td></td>
<td></td>
<td>3 \times 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>22*</td>
<td></td>
<td></td>
<td>-0.0043</td>
<td></td>
</tr>
<tr>
<td>1*</td>
<td></td>
<td></td>
<td>2 \times 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td>9 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>24*</td>
<td></td>
<td></td>
<td>3 \times 10^{-3}</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.31 illustrates the Michael acceptors which participated in a Michael addition reaction as well as the products which had LCMS/MS daughter scan. For each Michael acceptor and Michael product a found mass generated from the LCMS/MS daughter scan and the predicted fragments are listed. Some compounds have multiple
daughter ions found and are listed with the greatest ion listed first. These results confirm that suspected Michael products were formed.

**Table 2.31. Reactant and Michael Product Parent Compound and Fragments.** For each compound, a found fragment or daughter ion was found *via* LCMS/MS. From the found daughter ion, a predicted ion was made. Some compounds have multiple ion masses. For each mass found two ions are listed, the fragment which best matches the found daughter ion mass (top) and the counterpart to that ion (bottom).

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Michael Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7</strong></td>
<td><strong>27</strong></td>
</tr>
<tr>
<td>Found: 7</td>
<td>Predicted: 27</td>
</tr>
<tr>
<td>Exact Mass: 122.89</td>
<td>Exact Mass: 123.0</td>
</tr>
<tr>
<td>Exact Mass: 57.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>11</strong></th>
<th><strong>12</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Found: 102.96</td>
<td>Predicted: 104.83</td>
</tr>
<tr>
<td>Exact Mass: 103.1</td>
<td></td>
</tr>
<tr>
<td>Exact Mass: 105.0</td>
<td></td>
</tr>
<tr>
<td>Exact Mass: 105.0</td>
<td></td>
</tr>
<tr>
<td>Exact Mass: 213.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>12</strong></th>
<th><strong>106.97</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Found: 182.12</td>
<td>Predicted: 106.0</td>
</tr>
<tr>
<td>Exact Mass: 27.0</td>
<td></td>
</tr>
<tr>
<td>Exact Mass: 26.0</td>
<td></td>
</tr>
<tr>
<td>131.02</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>131.11</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>102.94</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>102.94</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>106.44</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>132.06</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>132.06</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>182.10</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>182.10</td>
<td><img src="image_url" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>

**Exact Masses:**
- 131.0
- 78.0
- 103.1
- 106.0
- 131.11
- 105.0
- 182.10
- 199.1
- 120.0
<table>
<thead>
<tr>
<th></th>
<th>103.01</th>
<th>210.25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 106.0</td>
<td>Exact Mass: 210.1</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 103.1</td>
<td>Exact Mass: 109.0</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>182.14</td>
<td>105.97</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td>14</td>
<td>Exact Mass: 181.1</td>
<td>Exact Mass: 105.0</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 28.0</td>
<td>Exact Mass: 214.1</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td>16</td>
<td>133.02</td>
<td>104.95</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 133.1</td>
<td>Exact Mass: 105.0</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td>16</td>
<td>104.96</td>
<td>161.05</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 105.0</td>
<td>Exact Mass: 161.1</td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td>16</td>
<td>161.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td><img src="" alt="Molecule" /></td>
<td><img src="" alt="Molecule" /></td>
</tr>
<tr>
<td></td>
<td>Exact Mass: 161.1</td>
<td>Exact Mass: 77.0</td>
</tr>
<tr>
<td>Number</td>
<td>Mass</td>
<td>Structure 1</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>17</td>
<td>118.05</td>
<td><img src="image1.png" alt="Structure 1" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image3.png" alt="Structure 1" /></td>
</tr>
<tr>
<td>17</td>
<td>130.98</td>
<td><img src="image5.png" alt="Structure 1" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image7.png" alt="Structure 1" /></td>
</tr>
<tr>
<td></td>
<td>103.03</td>
<td><img src="image9.png" alt="Structure 1" /></td>
</tr>
<tr>
<td>18</td>
<td>134.86</td>
<td><img src="image11.png" alt="Structure 1" /></td>
</tr>
<tr>
<td></td>
<td>103.04</td>
<td><img src="image13.png" alt="Structure 1" /></td>
</tr>
<tr>
<td></td>
<td>131.04</td>
<td><img src="image15.png" alt="Structure 1" /></td>
</tr>
<tr>
<td>Exact Mass</td>
<td>Exact Mass</td>
<td>Exact Mass</td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>262.98</td>
<td>268.1</td>
<td>121.97</td>
</tr>
<tr>
<td>105.92</td>
<td>78.0</td>
<td>336.1</td>
</tr>
<tr>
<td>106.92</td>
<td>106.0</td>
<td>350.1</td>
</tr>
</tbody>
</table>

Exact Mass: 120.0

Exact Mass: 106.0
2.4 Synthesis of Michael Products and Acceptors

Scheme 2-3 illustrates the synthesis for compounds 27 and 28 and 29. These compounds were synthesized via a Michael addition reaction in the presence of NEt$_3$, base. These compounds were produced from Michael acceptors, 7, 8, and 9, with Michael donor, thiophenol. For compound 27 R = H, compound 28 R = CH$_3$, and compound 29 R = Ph.

Scheme 2-3. Synthesis of 4[(Phenylmethyl)thio]-2-butanone (27), 4-Thiobenzyl-2-pentanone (28) and 4-Phenyl-4-(phenylthio)-2-butanone. Reagents and conditions: a) Thiophenol, MeOH, Net$_3$, rt

Most Michael acceptors were able to be synthesized in a one-step reaction. Other reactions needed an additional step in order to achieve the final compound. Compounds were synthesized with a Claisen-Schmidt condensation or a variation of that reaction.

Scheme 2-4 illustrates the general synthesis for the chalcones 11, 12, 13, 14, 15, 16, 17, and 18. The compound with its respective starting materials can be found in Table 2.32. Each compound has two starting materials; one being an aldehyde and the other being an acetyl.
Scheme 2-4. General Synthesis for Various Chalcones. Reagents and Conditions: \(a\) Piperidine, MeOH, reflux, or EtOH, 40% aq. NaOH, 0 °C.

Table 2.32. Final Compound and Starting Material with Corresponding Method. The following final compounds were synthesized from one of two methods. The final compound is listed with its starting materials and reaction method.

<table>
<thead>
<tr>
<th>Final compound</th>
<th>Aldehyde</th>
<th>Acetyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R \equiv \text{O} \equiv R')</td>
<td>(R \equiv \text{C} \equiv \text{H})</td>
<td>(R' \equiv \text{C} \equiv \text{O})</td>
</tr>
<tr>
<td>Reagents &amp; Conditions: Piperidine, MeOH, reflux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>Benzaldehyde</td>
<td>Acetylphenone</td>
</tr>
<tr>
<td>(12)</td>
<td>Benzaldehyde</td>
<td>4-Acetylpyridine</td>
</tr>
<tr>
<td>(13)</td>
<td>Benzaldehyde</td>
<td>3-Acetylpyridine</td>
</tr>
<tr>
<td>Reagents &amp; Conditions: EtOH, 40% aq. NaOH, 0 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(16)</td>
<td>3-Methoxyaldehyde</td>
<td>Acetophenone</td>
</tr>
<tr>
<td>(17)</td>
<td>Benzaldehyde</td>
<td>4-Methoxyacetophenone</td>
</tr>
<tr>
<td>(18)</td>
<td>Benzaldehyde</td>
<td>3-Methoxyacetophenone</td>
</tr>
</tbody>
</table>
Scheme 2-5 illustrates the synthesis for compound \textbf{19}. Its synthesis is similar to that of compound \textbf{1}, first a formylation reaction is achieved with a Vilsmeier-Hack reaction using POCl$_3$ and DMF to form compound \textbf{27}. The formylated indole is then condensed with benzaldehyde to form the desired compound \textbf{19}.

![Scheme 2-5. Synthesis of 3-(5-methoxy, 2-methyl-1H-indol-3-yl)-1-(4-benzyl)-2-propen-1-one. Reagents and conditions: (a) POCl$_3$, DMF, 0 °C, (b) benzaldehyde, MeOH, reflux.]

Scheme 2-6 shows the synthesis of compound \textbf{1} (MOMIPP). To achieve the final compound, a two-step reaction is required. First a formylation followed by the standard condensation reaction.\textsuperscript{5,6,7} The formylation is achieved by a Vilsmeier-Hack reaction using POCl$_3$ and DMF to form \textbf{27}. The formylated indole is then condensed with 4-acetylpyridine to form the desired compound \textbf{1}.

![Scheme 2-6. Synthesis of 3-(5-methoxy, 2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (MOMIPP). Reagents and conditions: (a) POCl$_3$, DMF, 0 °C, (b) 4-acetylpyridine, MeOH, reflux.]

181
Scheme 2-7 illustrated the synthesis of a diphenyl amide compound 26. The amide bond is created by treating analine with an acid chloride and TEA. The TEA may help facilitate the formation of the amide bond by abstracting a proton but it is also used to neutralize the HCl that is generated as a by-product from this reaction.

![Scheme 2-7](image)

**Scheme 2-7 Synthesis of 3-Diphenyl-2-propenamide.** Reagents and Conditions: a) Analine, NEt₃, 0 ºC to rt.

2.5 Conclusion

For the synthesis of Michael addition products using thiophenol donors, MeOH with a base such as NEt₃ can be deployed for quick and efficient reaction. Data collected from the Michael addition study via HPLC/LCMS, provided SAR applicable to the reactant’s chemical properties and to potential behaviors within biological matrices. There is a significant steric impediment at the 4-position of the α,β-unsaturated ketone. This phenomenon is evident in the difference between a methyl group and a phenyl group. The methyl has a spherical electron cloud which can hinder the attack of the nucleophile in any direction, whereas the phenyl group is planar allowing for a nucleophile attack along certain trajectories. Also, the phenyl group is in conjugation with the carbon-carbon double bond which can assist Michael addition. In general, substituents next to the carbonyl are well tolerated. This is not only seen by the data collected during the study, but also by the large groups next to the carbonyl seen in the drug molecules surveyed from the literature in chapter 1.
Pyridine rings are well tolerated on either side of the α,β-unsaturated ketone whereas indoles are highly unacceptable at the 4-position, except for one case with MOFLIPP which contains a trifluoromethyl group. This is significant when comparing the Michael reactivity of MOMIPP and MOFLIPP. MOMIPP contains a methyl group whereas MOFLIPP contains a trifluoromethyl group. MOMIPP is considered essentially non-reactive with a rate constant of $2 \times 10^{-3}$ or 0.002 per h whereas MOFLIPP is considered to have low Michael reactivity with a rate constant of 0.004 per h, double that of MOMIPP. Even though there are greater differences which can be found between other substitutions, the aforementioned rate difference allowed Michael addition product to be observed during MOFLIPP reactions whereas none could be found during MOMIPP reactions. Within the biological setting, MOMIPP is a methuosis-inducer in that it causes vacuolization followed by non-apoptotic cell death, whereas MOFLIPP causes apoptotic cell death with no formation of vacuoles. The noted difference in chemical behavior thus supports the hypothesis that these compounds target different pathways or that there are multiple pathways which are differentially triggered in order to induce methuosis versus apoptosis.

2.6 Experimental

2.6.1 Materials and Methods

All reagents and solvents were purchased through commercial sources (Sigma Aldrich or Fisher Scientific) and were used without further purification. All reactions were monitored by Thin Layer Chromatography (TLC) on F$_{254}$ plates, Baker-flex and visualized on 254 nm UV light. Normal phase flash chromatography was performed using silica gel (230-400 mesh). Samples to be purified by column chromatography were loaded onto silica either
by dissolving in minimal amounts of solvent and loading directly onto the column, or dissolved in minimal amount of solvent in a silica slurry, which was then evaporated and dry loaded. Appropriate fractions were combined and distilled \textit{in vacuo}, and then dried by a vacuum pump (0.5 mm Hg) overnight. Melting points were performed on an Electrothermal digital melting point apparatus. \textsuperscript{1}H NMR and \textsuperscript{13}C NMR experiments were recorded on a 600 MHz Bruker Avance spectrometer. Samples were referenced to the solvent residual peak. \textsuperscript{1}H NMR coupling constants ($J$ values) were expressed in hertz (Hz) using the following designations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, m = multiplet. Elemental analyses were performed by Atlantic Microlab (Norcross, GA).

4-(Phenylthio)-2-butanone (\textit{27})

3-buten-2-one (0.12 mL, 1.43 mmol), thiophenol (0.14 mL, 1.43 mmol) and triethylamine (0.1 mL, 0.715 mmol) were stirred in methanol (20 mL) for 40 min. Additional 3-buten-2-one (0.06 mL 0.715 mmol) was added to react with the remaining thiophenol. The solvent was distilled \textit{in vacuo} and the residue was redissolved in DCM (25mL). The organic layer was washed with a solution of water (25 mL) and acetic acid (1 mL) and further dried over anhydrous sodium sulfate. The sample was concentrated \textit{in vacuo}. The yellow oil was purified using column chromatography with a gradient of 0%–20% to yield an yellow oil (0.21 g, 81%): TLC $R_f$ 0.52 in 20\% EtOAC in hexanes. \textsuperscript{1}H NMR (600 MHz, CDCl$_3$) $\delta$ 7.33 (m, 5H), 3.14 (t, 2H, $J=7.2$), 2.77 (t, 2H, $J=7.2$), 2.15 (s, 3H); \textsuperscript{13}C NMR (150 MHz, CDCl$_3$) $\delta$ 206.86, 134.38, 132.56, 129.17, 127.47, 38.39, 30.81, 21.16. Elemental analysis calcd for C$_{10}$H$_{12}$OS: C, 66.63; H, 6.71; Found: C, 66.84; H, 6.60.
4-(Phenylthio)-2-pentanone (28)

3-penten-2-one (0.12 mL, 1.19 mmol), thiophenol (0.12 mL, 1.19 mmol) and triethylamine (0.08, 0.595 mmol) were stirred in methanol (20 mL) for 40 min. Additional 3-penten-2-one (0.06 mL 0.595mmol) was added to react with the remaining thiophenol. The solvent was distilled in vacuo and the residue was redissolved in DCM (25mL). The organic layer was washed with a solution of water (25 mL) and acetic acid (1 mL) and further dried over anhydrous sodium sulfate. The sample was concentrated in vacuo. The colorless oil was purified using column chromatography with a gradient of 0%-20% ethyl acetate and hexanes to yield a colorless oil (0.32g, 84 %): TLC Rf 0.48 in 20% EtOAc in hexanes. 1H NMR (600 MHz, CDCl3) δ 7.39 (m, 5H), 3.69 (m, 1H), 2.60 (m, 2H), 2.15 (s, 3H), 1.27 (d, 3H, J=6.72); 13C NMR (150 MHz, CDCl3) δ 206.86, 134.38, 132.56, 129.17, 127.47, 50.52, 38.39, 30.81, 21.16. Elemental analysis calcd for C11H14OS: C, 68.00; H, 7.26; Found: C, 67.90; H, 7.20.

4-Phenyl-4-(phenylthio)-2-butanone (29)

Thiophenol (0.17 mL, 13.68 mmol) and TEA (0.1mL, 6.84 mmol) were dissolved in methanol (30 mL) at rt. 4-phenyl-3-buten-2-one (201.6 mg, 13.68 mmol) was added to the reaction mixture and was allowed to stir at rt for 40 min. The solvent was distilled in vacuo and the residue was redissolved in DCM (25mL). The organic layer was washed with a solution of water (25 mL) and acetic acid (1 mL) and further dried over anhydrous sodium sulfate. The sample was concentrated in vacuo. The yellow oil was purified using column chromatography with a gradient of 0%-20% EtOAc in hexane to yield a colorless oil.
trans-Chalcone (11)
Benzaldehyde (1.9 mmol, 200 mg) was dissolved in MeOH (8 mL). Acetopheone (2.87 mmol, 0.4 mL) and piperidine (2.83 mmol, 0.3 mL) were added to the reaction mixture and refluxed overnight. Upon completion, the reaction mixture was concentration in vacuo and column purified with a gradient of 20-40 % EtOAc in hexanes to yield a yellow solid (143 mg, 36%): TLC Rf 0.42 in 20% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.04-8.02 (m, 2H), 7.83-7.81 (d, 1H $J=15.72$ Hz), 7.66-7.65 (m, 2 H), 7.64-7.58 (m, 1 H), 7.56-7.50 (m, 3H), 7.45-7.42 (m, 3 H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 190.73, 145.01, 138.24, 135.01, 132.94,130.70, 129.11, 128.78, 128.65, 128.60, 122.21; mp 53.5-55 °C. Elemental Analysis calcd for C$_{15}$H$_{12}$O: C, 86.51; H, 5.81; Found: C, 85.69; H, 5.78.

3-phenyl-1(4-pyridinyl)-2-propen-1-one (12)
Benzaldehyde (18 mmol, 200 mg) was dissolved in MeOH (8 mL). 4-Acetylpyridine (28 mmol, 334 mg) and piperidine (2.8 mmol, 0.3 mL) was added to the solution and refluxed overnight. The reaction mixture was concentrated in vacuo to produce a red oil which was column purified with a gradient of 20-70% EtOAc in hexanes. The pure fractions were
collected and dried in vacuo to yield pale yellow crystals (86.6 mg, 23%). TLCC Rf 0.51
80% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.85-8.84 (d, 2H, J=4.02 Hz), 7.85-
7.82 (d, 1H, J=15.78 Hz), 7.78-7.77 (m, 2H), 7.66-7.64 (m, 2H), 7.46-7.38 (m, 4H): $^{13}$C
NMR (150 MHz, CDCl$_3$) δ 190.06, 150.97, 147.03, 144.52, 134.42, 131.38, 129.26, 128.85, 121.69, 121.32; mp 82-83.5 °C. Elemental Analysis calcd for CHNO; C, 80.36; H, 5.30; N, 6.69; Found: C, 80.10; H, 5.46; N, 6.59.

3-phenyl-1-(3-pyridinyl)-2-propen-1-one (13)

Benzaldehyde (18 mmol, 200 mg) was dissolved in MeOH (8 mL). 3-Acetylpyridine (28
mmol, 0.31 mL) and piperidine (2.8 mmol, 0.28 mL) were added to the solution and
refluxed for 48 h. The reaction mixture was concentrated in vacuo and column purified
with a gradient of 20-70 % EtOAc in hexanes. The pure fractions were dried in vacuo to
yield a yellow solid. The solid was recrystallized in EtOH and H$_2$O to yield pale yellow
crystals (60.1 mg, 15 %) TLC Rf 0.53 80% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$)
δ 9.24-9.23 (d, 1H, J= 1.56 Hz), 8.82-8.81 (dd, 1H, J$_1$=1.68 J$_2$=1.68 Hz), 8.31-8.29 (m, 1H), 7.87-7.84 (d, 1H J=15.66 Hz), 7.68-7.65 (m, 2H), 7.51-7.48 (d, 1H, J=15.72 Hz), 7.48-7.43 (m, 5H): $^{13}$C NMR (150 MHz, CDCl$_3$) δ 189.32, 153.34, 149.90, 146.21, 136.08, 133.65, 131.18, 129.23, 128.79, 123.79, 121.50; mp 81.5-83.1 °C. Elemental Analysis calcd for C$_{14}$H$_{11}$NO; C, 80.36; H, 5.30; N, 6.69; Found: C, 80.22; H, 5.42; N, 6.90.
3-(3-methoxyphenyl)-1-phenyl-2-propen-1-one (16)

3-Methoxyaldehyde (16 mmol, 241 mg) and acetophenone (18 mmol, 0.21 mL) was dissolved in EtOH (10 mL) and chilled to 0 °C. A 40% aq. NaOH solution (2 mL) was added to the solution dropwise. The reaction mixture was stirred at 0 °C for 1 h and then warmed to rt overnight. The reaction mixture was then poured into ice water (50 mL) and neutralized with HCl (1 mL). The water was extracted with EtOAc (100 mL). The organic layer was dried over anhydrous sodium sulfate, filter, and dried in vacuo to produce a yellow oil. The yellow oil was column purified with a gradient of 0-20% EtOAc in hexanes. The pure fractions were collected and dried in vacuo to yield a yellow oil (226.7 mg, 59%).

TLC Rf 0.49 20% EtOAc in hexanes. ¹H NMR (600 MHz, CDCl₃) δ 8.03-8.01 (m, 2H), 7.79-7.76 (d, 1H, J=15.66 Hz), 7.60-7.58 (m, 1H), 7.53-7.750 (m, 3H), 7.36-7.33 (m, 1H), 7.26-7.24 (m, 1H), 7.16-7.15 (t, 1H, J₁=2.1 Hz, J₂=1.92 Hz), 6.98-6.96 (m, 1H), 3.86 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 190.73, 160.08, 144.93, 138.32, 136.40, 132.96, 130.11, 128.78, 128.67, 122.54, 121.25, 116.46, 113.56, 55.51. Elemental Analysis calcd for C₁₆H₁₄O₂; C, 80.65; H, 5.92; Found: C, 80.34; H, 5.90.

1-(4-methoxyphenyl)-3-phenyl-2-propen-1-one (17)

Benzaldehyde (18 mmol, 200mg) and 4-methoxyacetopheone (20 mmol, 306.9 mg) was dissolved in EtOH (10 mL) and chilled to 0 °C. A 40% aq NaOH (2 mL) was added to the cooled solution dropwise. The reaction mixture was stirred at 0 °C for 1 h and then warmed to rt and stirred overnight. A precipitate formed and was filtered with H₂O to yield an off white solid (387.1 mg, 89%). TLC Rf 0.37 80% EtOAc in hexanes. ¹H NMR (600 MHz,
CDCl$_3$) $\delta$ 8.06-8.04 (m, 2 H), 7.82-7.79 (d, 1 H, $J$=15.66 Hz), 7.65-7.64 (m, 2 H), 7.57-7.54 (d, 1 H, $J$=15.66 Hz), 7.43-7.40 (m, 3 H), 7.00-6.98 (m, 2 H), 3.89 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 188.86, 163.56, 144.11, 135.21, 131.23, 130.97, 130.47, 129.07, 128.50, 122.00, 113.98, 55.65. Elemental Analysis calcd for C$_{16}$H$_{14}$O$_2$; C, 80.65; H, 5.92; Found: C, 80.36; H, 6.05.

1-(3-methoxyphenyl)-3-phenyl-2-propem-1-one (18)

Benzaldehyde (18 mmol, 200 mg) and 3-methoxyacetophenone (18 mmol0.25 mL) was dissolved in EtOH (10 mL) and chilled 0 ºC. A 40% aq NaOH solution (2 mL) was added to the reaction mixture dropwise and continued to stir at 0 ºC for 1 h. Then the reaction mixture was warmed to rt overnight. The reaction mixture was poured into ice water (50 mL) and neutralized with concentrated HCl (1 mL). The water was extracted with EtOAc (100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to a yellow oil. The oil was column purified with a gradient of 0-7% EtOAc in hexanes. The pure fractions were dried in vacuo to yield a yellow oil (387.1 mg, 89%). TLC $R_f$ 0.47 20% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$7.83-7.81 (d, 1 H, $J$=15.66 Hz), 7.66-7.64 (m, 2H), 7.62-7.60 (m, 1 H), 7.55-7.55 (m, 1H), 7.53-7.51 (d, 1 H, $J$=15.72 Hz), 7.44-7.41 (m, 4 H), 7.15-7.13 (m, 1 H), 3.89 (s, 3 H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$190.43, 160.04, 145.03, 139.73, 135.01, 130.71, 129.72, 129.11, 128.61, 122.24, 121.21, 119.48, 112.97, 55.64. Elemental Analysis calcd for C$_{16}$H$_{14}$O$_2$·0.1 H$_2$O; C, 80.04; H, 5.96; Found: C, 79.94; H, 6.02.
5-methoxy-2-methylindole-3-carboxyaldehyde\(^5\) (30)

A flask containing a mixture of POCl\(_3\) (10.7 mmol, 1 mL) and DMF (2 mL) stirred for 10 min at 0 °C under N\(_2\) (g). 2-Methyl-5-methoxyindole (1 g, 6.2 mmol) was dissolved in DMF (3 mL) and added to the stirred solution dropwise. The reaction mixture continues to stir at 0 °C for an additional 10 min and then warmed to rt where it stirred for an additional 30 min. The reaction mixture was poured into an ice cold 1 N NaOH solution (35 mL). A cream precipitate formed. The precipitate was filtered and washed with H\(_2\)O (35 mL) to yield a cream powdery solid (1.0481 g, 89%). TLC R\(_f\) 0.50 80 % EtOAc in hexanes. \(^1\)H NMR (600 MHz, CDCl\(_3\) \(\delta\) 10.14 (s, 1 H), 7.77-7.76 (d, 1 H, \(J=2.4\) Hz), 7.22-7.20 (d, 1 H, \(J=8.76\) Hz), 6.87-6.86 (dd, 1 H, \(J_1=2.52\) Hz, \(J_2=2.52\) Hz), 3.87 (s, 3 H), 3.50 (s, 3 H), 2.71 (s, 3 H); \(^{13}\)C NMR (150 MHz, CDCl\(_3\) \(\delta\) 184.61, 156.62, 147.10, 129.73, 126.89, 115.05, 113.58, 111.58, 102.95, 55.93, 51.05, 12.36. mp 185-187 °C.

3-(5-methoxy, 2-methyl-1\(H\)-indol-3-yl)-1-(4-benzyl)-2-propen-1-one (19)

5-methoxy-2-methylindole-3-carboxyaldehyde\(^5\) (30, 4.23 mmol, 81.2 mg) was dissolved in MeOH (8 mL). Acetophenone (6.3 mmol, 0.1 mL) and piperidine (6.3 mmol, 0.1 mL) was added to the solution and refluxed overnight. The reaction was cooled to rt at which point a precipitate formed and then the reaction mixture was cooled to 0 °C to further precipitate. The precipitate was filter and the filtrate was column purified with a gradient of 0-50% EtOAc in hexanes. The pure fractions were dried in vacuo to yield an orange solid (92.3 mg, 75%). TLC R\(_f\) 0.53 in 80% EtOAc in hexanes. \(^1\)H NMR (600 MHz, CDCl\(_3\) \(\delta\) 8.42 (s, 1H), 8.17-8.15 (d, 1 H, \(J=15.42\) Hz), 8.07-8.05 (m, 2 H), 7.58-7.56 (m, 1 H), 7.53-7.50 (m,
2 H, 7.49-7.47 (d, 1 H, J=15.42 Hz), 7.43-7.42 (d, 1 H, J=2.34 Hz), 7.25-7.24 (d, 1 H, J=8.7 Hz), 6.89-6.87 (dd, 1 H, J1=2.4 Hz, J2=2.4 Hz), 3.92 (s, 3 H), 2.59 (s, 3 H); 13C NMR (150 MHz, CDCl3) δ 190.92, 155.59, 142.57, 139.31, 138.32, 132.15, 130.77, 128.55, 128.28, 127.18, 116.16, 111.59, 111.23, 110.64, 103.95, 56.10, 12.75.

3-(5-Methoxy, 2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (MOMIPP; 1)

To a dried two-neck flask purged with N2 (g), 5-methoxy-2-methylindole-3-carboxyaldehyde5 (30 1.05 g, 5.53 mmol) was dissolved in anhydrous methanol (30 mL). 4-Acetylpyrdine (1.37 g, 11 mmol) and piperdine (968 mg, 11 mmol) were added and the mixture was allowed to reflux for 12 hr. An orange precipitate slowly began to form. Upon completion, the precipitate was collected by vacuum filtration, washed with chilled methanol and dried in vacuo yielding an orange solid (1.42 g, 88%): TLC Rf 0.25 in EtOAc in hexanes (4:1). 1H NMR (600 MHz, d6-DMSO) δ 11.89 (s, 1H), 8.81-8.80 (d, 2H, J=5.82 Hz), 8.05-8.07 (d, 1H, J=14.34 Hz), 7.93-7.94 (d, 2H, J=5.82 Hz), 7.43 (s, 1H), 7.37-7.34 (d, 1H, J= 16.62 Hz), 7.32-7.31 (d, 1H, J= 8.82 Hz), 6.83-9.85 (d, 1H, J=8.64 Hz) 3.86 (s, 3H), 2.57 (s, 3H); 13C NMR (150 MHz, d6-DMSO) δ 188.52, 155.65, 151.08, 146.26, 145.56, 140.06, 131.46, 127.05, 121.89, 113.26, 112.74, 111.38, 109.77, 103.97, 56.04, 12.62; mp 254-256°C (lit.5 mp 252-256°C). Elemental Analysis calcd for C18H16N2O2: C, 73.95; H, 5.52; N, 9.58; Found: C, 73.87; H, 5.52; N, 9.53.
3-Diphenyl-2-propenamide (26)

Under N₂ (g), Analine (3.22 mmol, 300 mg) and NEt₃ (0.94 mL) was dissolved in dried DCM (5 mL) and stirred for 15 min and chilled to 0 °C. 3-Phenyl-2-propenoyl chloride (4.06 mmol, 715 mg) was dissolved in dry DCM (1.5 mL). The acid chloride solution was added to the chilled analine solution dropwise. The reaction mixture was warmed to rt and stirred under N₂ (g) overnight. The reaction mixture was concentrated in vacuo and suspended in 1 N HCl solution and extracted with EtOAc (90 mL). The organic layer was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to a brown oil. The oil was column purified with a gradient of 0-10% hexanes in DCM and then 0-75% EtOAc in DCM. The pure fractions were collected and dried in vacuo to yield a yellow oil (145 mg, 20%). TLC Rf 0.29 20% EtOAc in hexanes; ¹H NMR (600 MHz, CDCl₃) δ 7.77-7.75 (d, 1H, J=15.48 Hz), 7.64-7.63 (d, 1H, J=6 Hz), 7.53-7.52 (m, 2 H), 7.44 (s, 1 H), 7.40-7.34 (m, 5 H), 7.14-7.12 (t, 1 H, J₁=7.14 Hz, J₂=7.2 Hz), 6.58-6.55 (d, 1 H, J=15.48 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 142.60, 134.72, 130.14, 129.24, 129.03, 128.11, 124.58, 120.05; Elemental Analysis calcd for C₁₅H₁₃NO: C, 80.69; H, 5.87; N, 6.27; Found : C, 80.52; H, 5.98; N, 6.35.
Chapter 3

Metabolite Synthesis

3.1 Introduction

As described in Chapter 1, understanding the metabolism of a potential drug candidate is a key component in drug development. The metabolites for 1 were discovered from cultured human U251 glioblastoma cells after analysis by LCMS and LCMS/MS. Figure 6 from Chapter 1 depicts the results for the metabolite studies. Three metabolites, Figure 1, were identified, two reflecting partial reduction of the α,β unsaturated ketone and the last a full reduction of the α,β unsaturated ketone. It is important to know the biological effect of these metabolites on the cell to understand the toxicity of such metabolites as well as the potential mode of action for the parent molecule.
Figure 3-1. MOMIPP Parent Molecule and Metabolites. Compound 1 is the parent molecule, MOMIPP. M1, M2A, and M2B are the metabolites found in the study described in Chapter 1.4 Metabolism. M2A and M2B are partially reduced versions of MOMIPP whereas M1 is a fully reduced version of MOMIPP.

Several possibilities can be examined to synthesize the metabolites. The parent compound, MOMIPP, can be reacted with reducing agents such as sodium borohydride and lithium aluminum hydride to achieve partial reduction of the α,β-unsaturated ketone.\textsuperscript{16} To achieve full reduction catalytic hydrogenation was explored using catalysts such as palladium.\textsuperscript{16} Oxidation reagents were also used to form partially reduced forms of MOMIPP from the fully-reduced form.\textsuperscript{17}
Figure 3-2. Synthetic Strategies for Synthesis of MOMIPP Metabolites. Reductions can include reagents such as sodium borohydride, LAH, and catalytic hydrogenation. The fully reduced version may be oxidized with reagents such as PDC. A standard molecule trans-4-phenyl-3-buten-2-one (R=Ph and R' = CH$_3$) was used for model reactions which was later applied to MOMIPP (R=5-methoxy-2-methyl-1H-indol-3-yl and R' = 4-pyridinyl).

3.2 Reductions

A standard compound, *trans*-4-phenyl-3-buten-2-one (R=Ph, R' = Me in Figure 3-2) was used for model reactions to determine which conditions would yield which reduced form most effectively. The standard compound was subjected to sodium borohydride and lithium aluminum hydride. Sodium borohydride reduces the ketone to an alcohol whereas lithium aluminum hydride fully reduces the $\alpha,\beta$-unsaturated ketone to a saturated secondary alcohol.$^{16}$ To achieve the selective carbon-carbon double bond reduction, catalytic hydrogenation using palladium on carbon was attempted.$^{16}$ Palladium catalyzed hydrogenation can be used to selectively reduce carbon-carbon bonds as well as reducing both the carbon-carbon double bond and the ketone of the $\alpha,\beta$-unsaturated ketone.$^{16}$ Once these techniques were tested on the standard compound, the methods were then adapted to the desired compound, MOMIPP.
3.2.1 Results and Discussion

The standard compound, trans-4-phenyl-3-buten-2-one, was initially subjected to mild reducing agents to determine the degree of reduction. Table 3.1 depicts the results with the starting material, reagent and then resulting product. Sodium borohydride partially reduced the standard compound, only reducing the ketone to an alcohol. LAH fully reduced the standard compound. Palladium on carbon catalyzed hydrogenation only reduced the carbon-carbon double-bond.

Table 3.1. Reduction Reagent and Product for Standard. When the standard compound is treated with sodium borohydride the ketone was reduced to a secondary alcohol (3). When treated with lithium aluminum hydride both the ketone and the carbon-carbon double bond were reduced (4). Finally when catalytically hydrogenated, only the carbon-carbon double bond was reduced to form a saturated ketone (5).

<table>
<thead>
<tr>
<th>Starting compound</th>
<th>Reagent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>Sodium borohydride</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>LAH</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>Pd/C hydrogenation</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

When these methods were applied to the desired compound, MOMIPP, the results were much different (Table 3.2). Neither sodium borohydride nor LAH produced any of the metabolites. These reaction product mixtures were too complex to determine what was being produced. When MOMIPP was subjected to hydrogenation with palladium on carbon as the catalyst, a mixture of all three reduced versions formed, both partially reduced as well as the fully reduced. Initially a mixture of DMSO and MeOH was used as the solvent,
which differed from the standard method which only used MeOH. The use of DMSO was for enhanced solubility. However, since it is a potentially reactive compound under these conditions, it could have some role in the mixed results. The solvent was then changed to a ratio of 2:1 DCM: MeOH. The starting material was soluble and this time the reaction yielded a single product, namely the fully reduced version. The fully reduced MOMIPP metabolite form was biologically tested and was found to be inactive in producing methuosis as well as non-toxic to the cells.

**Table 3.2. Reducing Reagent and Product for MOMIPP.** When MOMIPP was treated with sodium borohydride and LAH the reactions were complex and did not lead to desired product. However, when MOMIPP was catalytically hydrogenated, the fully reduced metabolite (M1) was produced when DCM/MeOH was used.

<table>
<thead>
<tr>
<th>Starting compound</th>
<th>Reagent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium borohydride</td>
<td>Complex Reaction</td>
<td></td>
</tr>
<tr>
<td>LAH</td>
<td>Complex reaction</td>
<td></td>
</tr>
<tr>
<td>Pd/C hydrogenation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO/ MeOH</td>
<td>Mixture of Desired Products</td>
<td></td>
</tr>
<tr>
<td>DCM/MeOH</td>
<td>M1</td>
<td></td>
</tr>
</tbody>
</table>

The initial formation of all three metabolites from the hydrogenation with the presence of DMSO demonstrates that either there is a succession in reduction and/or the partially reduced versions of MOMIPP are unstable. It is important to note that the work-up for the hydrogenation as compared to the other reducing reagents is far less. For the hydrogenation, a simple filtration over Celite yielded a pure fully reduced MOMIPP, whereas the other reducing agents needed some kind of purification step (s). These
purification steps could be giving the possible unstable, partially reduced MOMIPP a chance to decompose to unrecognizable compounds.

To determine if this was a possibility a polymer borohydride was used to react and then simply filter away with little to no purification needed. When the standard compound was treated with the polymer borohydride the reaction was very slow and never went to completion, even when it was accelerated with heat. This slow reaction may have been ideal for MOMIPP, but when tested there was no promising formation of product and this method was not further pursued.

3.3 Oxidations

Once producing the fully reduced metabolite of MOMIPP, alternative reactions were conceptualized via starting with the fully reduced metabolite and oxidizing to achieve partially reduced metabolites (Figure 3-1). Pyridinium dichromate (PDC) was found to be a suitable oxidizing agent due to the reagents insolubility in DCM while retaining the chemical reactivity. This provides an opportunity to isolate metabolites suspected of having instability via more gentle reaction workups.

3.3.1 Results and Discussion

When the standard fully reduced compound was subjected to PDC, the alcohol was converted to a ketone at a slow rate. Due to the suspected instability of MOMIPP metabolites, reaction conditions were not accelerated. Differing from the results produced
by the standard compound, fully reduced MOMIPP treated with PDC did not produce the partially reduced alcohol at any ratio. In addition, the PDC seemingly degraded the starting material.

3.4 Conclusion

The standard compound served as a useful tool in determining reaction conditions and potential product outcomes which were also supported by the literature. However, it did not demonstrate how MOMIPP would behave because most reactions did not produce predicted outcomes. It seems that the metabolism may go through a sequence of reductions from partially reduced to fully reduced with the partially reduced metabolites having some stability issues when attempts are made to synthesis and isolate them as distinct entities. Future directions for metabolites could include the use of enzymatic reductions to produce some if not all of the metabolites.

3.5 Experimental

3.5.1 Materials and Methods

All reagents and solvents were purchased through commercial sources (Sigma Aldrich or Fisher Scientific) and were used without further purification. All reactions were monitored by Thin Layer Chromatography (TLC) on F<sub>254</sub> plates, Baker-flex and visualized on 254 nm UV light. Normal phase flash chromatography was performed using silica gel (230-400
mesh). Samples to be purified by column chromatography were loaded onto silica either by dissolving in minimal amounts of solvent and loading directly onto the column, or dissolved in minimal amount of solvent in a silica slurry, which was then evaporated and dry loaded. Appropriate fractions were combined and distilled in vacuo, and then dried by a vacuum pump (0.5 mm Hg) overnight. Melting points were performed on an Electrothermal digital melting point apparatus. \(^1\)H NMR and \(^1\)C NMR experiments were recorded on a 600 MHz Bruker Avance spectrometer. Samples were referenced to the solvent residual peak. \(^1\)H NMR coupling constants (\(J\) values) were expressed in hertz (Hz) using the following designations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, m = multiplet. Elemental analyses were performed by Atlantic Microlab (Norcross, GA).

**5-methoxy-2-methylindole-3-carboxyaldehyde\(^5\) (30)**

A flask containing a mixture of POCl\(_3\) (10.7 mmol, 1 mL) and DMF (2mL) stirred for 10 min at 0 ºC under N\(_2\) (g). 2-Methyl-5-methoxyindole (1 g, 6.2 mmol) was dissolved in DMF (3 mL) and added to the stirred solution dropwise. The reaction mixture continues to stir at 0 ºC for an additional 10 min and then warmed to rt where it stirred for an addition 30 min. The reaction mixture was poured into an ice cold 1 N NaOH solution (35 mL). A cream precipitate formed. The precipitate was filtered and washed with H\(_2\)O (35 mL) to yield a cream powdery solid (1.0481 g, 89%). TLC R\(_f\) 0.50 80 % EtOAc in hexanes. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 10.14 (s, 1 H), 7.77-7.76 ( d, 1H, \(J = 2.4\) Hz), 7.22-7.20 (d, 1H, \(J =8.76\) Hz), 6.87-6.86 (dd, 1 H, \(J_1=2.52\) Hz, \(J_2=2.52\) Hz), 3.87 (s, 3 H), 3.50 (s, 3 H),
2.71 (s, 3 H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 184.61, 156.62, 147.10, 129.73, 126.89, 115.05, 113.58, 111.58, 102.95, 55.93, 51.05, 12.36. mp 185-187 ºC.

3-(5-Methoxy, 2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propan-1-one (MOMIPP; 1)

To a dried two-neck flask purged with N$_2$ (g), 5-methoxy-2-methylindole-3-carboxyaldehyde (1.05 g, 5.53 mmol) was dissolved in anhydrous methanol (30 mL). 4-Acetylpyridine (1.37 g, 11 mmol) and piperdine (968 mg, 11 mmol) were added and the mixture was allowed to reflux for 12 hr. An orange precipitate slowly began to form. Upon completion, the precipitate was collected by vacuum filtration, washed with chilled MeOH and dried in vacuo yielding an orange solid (1.42 g, 88%): TLC R$_f$ 0.25 20% EtOAC in hexanes. $^1$H NMR (600 MHz, $d_6$-DMSO) δ 11.89 (s, 1H), 8.81-8.80 (d, 2H, $J=5.82$ Hz), 8.05-8.07 (d, 1H, $J=14.34$ Hz), 7.93-7.94 (d, 2H, $J=5.82$ Hz), 7.43 (s, 1H), 7.37-7.34 (d, 1H, $J= 16.62$ Hz), 7.32-7.31 (d, 1H, $J= 8.82$ Hz), 6.83-9.85 (d, 1H, $J=8.64$ Hz) 3.86 (s, 3H), 2.57 (s, 3H); $^{13}$C NMR (150 MHz, $d_6$-DMSO) δ 188.52, 155.65, 151.08, 146.26, 145.56, 140.06, 131.46, 127.05, 121.89, 113.26, 112.74, 111.38, 109.77, 103.97, 56.04, 12.62; mp 254-256°C (lit.$^1$ mp 252-256°C). Elemental Analysis calcd for C$_{18}$H$_{16}$N$_2$O$_2$: C, 73.95; H, 5.52; N, 9.58; Found: C, 73.87; H, 5.52; N, 9.53.

3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propan-1-ol (M1)

3-(5-Methoxy, 2-methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (74.1mg, 0.25 mmol) was added to a mixture of MeOH and DCM (10 mL; 66%, MeOH in DCM) and
refluxed for 30 min. The remaining solid was filtered and to the filtrate was added palladium on carbon (12.7 mg). The reaction mixture attached to a PAPR hydrogenator and reacted under 35 psi H₂ for 16 hr. The mixture was filtered over Celite and concentrated in vacuo to yield a pale yellow oil (42.3 mg, 56%). TLC R₇ = 0.41 in 5% MeOH in DCM. 

^1^H NMR (600 MHz, CDCl₃) δ 8.57-8.56 (d, 2H, J=4.92 Hz), 7.70 (s, 1H), 7.28-7.27 (d, 2H, J=4.5 Hz), 7.19-7.17 (d, 1H, J=8.64 Hz), 6.95-6.94 (d, 1H, J=2.4 Hz), 6.80-6.78 (dd, 1H, J₁= 5.52 Hz, J₂=5.28 Hz), 4.74-4.72 (t, 1H, J=6.3 Hz), 3.86 (s, 3H), 2.88-2.83 (m, 2H), 2.37 (s, 3H), 2.08-2.05 (m, 2H). ^13^C NMR (150 MHz, CDCl₃) 153.9, 153.8, 149.8, 132.1, 130.4, 129.0, 121.0, 110.89, 110.49, 110.46, 100.6, 56.0, 41.0, 39.1, 20.1, 11.8. Elemental analysis calcd for C₁₈H₂₀N₂O₂·0.65 DMSO C: 66.82, H: 6.94, N: 8.07; Found C: 67.01;H: 6.99; N: 7.82.

**4-Phenyl-3-buten-2-ol (3)**

Sodium borohydride (79 mg, 2.0 mmol) was dissolved in EtOH (3 mL) and chilled in an ice bath. 4-Phenyl-3-buten-2-one (306.1 mg, 2.1 mmol) was dissolved in 3mL EtOH and added to the chilled sodium borohydride solution dropwise. The reaction mixture was chilled for 20 min and then brought to room temperature for 2 h. The reaction mixture was then diluted with water (30 mL) and extracted with ethyl ether (30 mL, 3x). The organic layer was washed with brine (40 mL) and dried over anhydrous sodium sulfate. The dried organic layer was concentrate in vacuo to produce a crude yellow oil. The crude oil was column purified with a gradient of 0-20% EtOAc in hexanes to yield a yellow oil (176.4 mg, 57%). TLC R₇ 0.25 in 20% EtOAc in hexanes ^1^H NMR (600 MHz, CDCl₃) 7.39-7.38 (m, 2H), 7.33-7.31 (m, 2H), 7.28-7.22 (m, 1H), 4.52-4.47 (m, 1H), 1.37-1.36 (d, 3H, J=6.7
Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) 136.8, 133.7, 129.6, 128.7, 127.8, 126.6, 69.1, 45.3, 30.2, 29.9, 23.6. Elemental Analysis calcd for C$_{10}$H$_{12}$O·0.45 H$_2$O C, 76.89; H, 8.32; C, 76.90; H, 8.16.

**4-Phenyl-3-butan-2-ol (4)**

Lithium aluminum hydride (2 mL, 2 mmol) in THF (2 mL) was chilled in an ice bath. 4-Phenyl-3-buten-2-one (268.8 mg, 1.8 mmol) was dissolved in THF (5 mL) and added dropwise to the chilled LAH solution. The reaction was brought to rt after 1 h. The reaction mixture was then quenched with 0.5 N HCl (30mL) and extracted with EtOAc (30 mL). The organic layer was washed with saturated sodium bicarbonate (30 mL) and brine (30 mL) and then dried over anhydrous sodium sulfate. The dried organic layer was concentrated in vacuo to produce a crude yellow oil. The oil was column purified with a gradient of 0-20% EtOAc in hexanes to yield a yellow oil (206.8 mg, 75%) TLC $R_f$ = 0.30 in 20% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$) 7.30-7.27 (m, 2H), 7.21-7.18 (m, 3H), 3.85-3.82 (m, 1H), 2.76-2.73 (m, 1H), 2.7-2.65 (m, 1H), 1.81-1.75 (m, 2H), 1.24-1.23 (d, 3H, $J$=3.42 Hz); $^{13}$C NMR (150 MHz, CDCl$_3$) 142.2, 128.6, 128.5, 128.4, 126.3, 126.0, 67.7, 41.0, 32.3, 23.8. Elemental Analysis calcd for C$_{10}$H$_{14}$O·0.45 EtOAc C, 79.03; H, 9.39; Found: C, 79.00; H, 9.00.

**4-Phenyl-3-butan-2-one (5)**

4-phenyl-3-buten-2-one (165.3 mg, 1.1 mmol) was dissolved in EtOAc (15 mL) and chilled in an ice bath for 10-15 min. Palladium on carbon (50 mg) was added and attached to
hydrogenator for 5 h. The Pd/C was filtered over Celite and concentrated in vacuo to a crude colorless oil. The oil was column purified with a gradient of 0-20% EtOAc in hexanes to produce a colorless oil (93.3 mg, 56%). TLC R$_f$ 0.45 in 20% EtOAc in hexanes. $^1$H NMR (600 MHz, CDCl$_3$) 7.30-7.27 (m, 2H), 7.21-7.18 (m, 3H), 2.91-2.90 (t, 1H, $J_1=7.4$ Hz, $J_2=7.9$ Hz), 2.78-2.75 (t, 1H, $J_1=7.9$ Hz, $J_2=7.4$ Hz), 2.14 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) 208.1, 141.1, 128.6, 128.4, 126.2, 45.3, 30.2, 29.8. Elemental Analysis calcd for C$_{10}$H$_{12}$O·0.05 H$_2$O C, 80.52; H, 8.18; Found: C, 80.62; H, 8.18.
Chapter 4

Indole-Based Chalcone Analogues

4.1 Introduction

Certain indole-based chalcones have been shown to induce methuosis. The molecular scaffold for these compounds is composed of indolyl and pyridinyl rings connected by an α,β-unsaturated ketone moiety. Substitutions on the indolyl moiety have been extensively studied.\textsuperscript{5,6,7} Substitutions on the pyridinyl ring have been only minimal. The ortho-, para- and meta-pyridine, pyrazine and phenyl have been synthesized and evaluated. The para-pyridine was the only analogue to show activity, making this arrangement seemingly essential for methuosis induction.

The effect of an external nitrogen placed in the para-position is not known. Removing the nitrogen from the ring alters its electronic effects which may affect the activity. Four analogues were conceptionalized: an acylated and boc-protected amine, free amine, and a nitro. The nitro analogue is the most electron withdrawing followed by the acylated, boc-protected and finally the free amine being the most electron donating analogue.
Table 4.1 Summary of Phenyl Analogue.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
</table>
| Acylated (3)              | \[
|                           | \text{NH} \quad \text{CO} \quad \text{O} \quad \text{H} \] |
| Boc-protected (4)         | \[
|                           | \text{NH} \quad \text{CO} \quad \text{O} \quad \text{H} \] |
| Free amine (5)            | \[
|                           | \text{NH} \quad \text{H} \] |
| Nitro (6)                 | \[
|                           | \text{NO} \quad \text{O} \quad \text{H} \] |

4.2 Synthesis of Phenyl Derivatives

These compounds can be synthesized in one to three steps. Their syntheses began with the acetophenone compounds having either an amine or a nitro group at position 4 of the ring (Scheme 4-1). Once the acetophenone intermediates were prepared, they were condensed with 5-methoxy-2-methylindole-3-carboxyaldehyde to achieve the final compounds 3, 4, and 6, with compound 5 (free amine) also requiring a subsequent deprotection of compound 4.

Scheme 4-1 depicts the synthesis of the acylated derivative 3 beginning with the acylation of 4-aminoacetophenone using TEA and acetic anhydride. Target compound 3 was then achieved by condensing the acylated intermediate 31 with 5-methoxy-2-methylindole-3-carboxyaldehyde using the standard procedure.\[^{5,6,7}\]
Scheme 4-1. Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(acetylaminophenyl)-2-propen-1-one. Reagents and conditions: (a) NEt₃, Acetic Anhydride, DCM, rt; (b) 5-methoxy-2-methylindole-3-carboxyaldehyde, piperidine, MeOH, reflux.³⁵,⁶,⁷

The boc-protected analogue 4 and the free amine analogue 4 syntheses are shown in Scheme 4-2. The 4-aminoacetophenone was initially protected with di-tert-butyl-dicarbonate to achieve the boc-protected intermediate 32. This intermediate was condensed with the aldehyde to produce the target compound 4. Compound 4 was then deprotected with TFA to produce free amine target 5.
Scheme 4-2. Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(N-boc-aminophenyl)-2-propen-1-one and 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1(aminophenyl)-2-propen-1-one. Reagents and conditions: (a) Di-tert-butyl-dicarbonate, THF, reflux; (b) 5-Methoxy-2-methylindole-3-carboxyaldehyde, piperidine, MeOH, reflux\(^5,6,7\); (c) TFA, MeOH, rt.

Scheme 4-3 illustrates the one step reaction required to synthesis compound 6.

There is no requirement for protection of the nitro group during the condensation step that has been shown in the previous schemes.

Scheme 4-3 Synthesis of 3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(nitrophenyl)-2-propen-1-one. Reagents and conditions: 5-methoxy-2methylindole-3-carboxyaldehyde, piperidine, MeOH, reflux\(^5,6,7\)
4.3 Biological Activity

These compounds were tested for their activity in producing vacuoles and eventually cell death (methuosis) against U251 human glioblastoma cells. These compounds were tested at 2.5 µM and 10 µM and at time points 4h, 24h, and 48h and examined by phase contrast microscopy, appendix A page 37. It was found that all of these analogues have a GI50 of > 10 µM and thus are essentially inactive. Our cut-off for this categorization is 10 µM since MOMIPP has significant activity is about 2 µM.

4.4 Conclusion

It has been shown that moving the nitrogen out of the ring dramatically decreases the activity toward inducing methuosis. This observation is maintained whether the nitrogen is assembled in an electron donating, neutral or electron withdrawing arrangement. It further supports the SAR where a pyridine ring with the nitrogen specifically located at the para-position appears to be necessary for methuosis induction.

4.5 Experimental

4.5.1 Materials and Method

Reagents and solvents were purchased from commercial sources (Sigma Aldrich or Fisher Scientific) and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on F254 plates, Baker-flex and visualized by 254 nm UV light.
Normal phase flash chromatography was performed using silica gel (230-400 mesh). Samples to be purified by column chromatography were loaded onto silica either by dissolving in minimal amounts of solvent and loading directly onto the column, or dissolved in minimal amount of solvent in a silica slurry, which was then evaporated and dry loaded. Appropriate fractions were combined and distilled in vacuo, and then dried by a vacuum pump (0.5 mm Hg) overnight. Melting points were performed on an Electrothermal digital melting point apparatus. $^1$H NMR and $^{13}$C NMR experiments were recorded on a 600 MHz Bruker Avance spectrometer. Samples were referenced to the solvent residual peak. $^1$H NMR coupling constants ($J$ values) were expressed in hertz (Hz) using the following designations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, m = multiplet. Elemental analyses were performed by Atlantic Microlab (Norcross, GA).

4-Acetylphenyl-acetamide (31)

4-Aminoacetophenone (501 mg, 3.7 mmol) was dissolved in DCM (8 mL). TEA (412 mg, 4.1 mmol) and acetic anhydride (415 mg, 4.1 mmol) was added at rt, under nitrogen and then stirred overnight. The reaction mixture was diluted with DCM (15 mL) and washed with 0.5 N HCl (25 mL) and brine (25 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to a white solid. The white solid was column purified with a gradient of 0-20% EtOAc in hexanes. The pure fractions were collected and dried to yield a white solid (337 mg, 51%) mp 172-174 °C TLC $R_f$ 0.37 20% EtOAc in hexanes. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.94-7.92 (d, 2H, $J$=8.7 Hz),
7.63-7.61 (d, 2H, J=6.5 Hz), 3.5 (s, 1H), 2.6 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) 197.2, 168.7, 142.4, 133.0, 129.9, 119.0, 51.0, 26.6, 25.0.

3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(acetylaminophenyl)-2-propen-1-one (3)

5-Methoxy-2-methylindole-3-carboxyaldehyde (200 mg, 1.05 mmole) was dissolved in MeOH (10 mL). Piperidine (90 mg, 1.05 mmole) and 4-acetylphenyl-acetamide (280 mg, 1.6 mmole) were added and the mixture was allowed to reflux for 12 h. An orange precipitate slowly begun to form. Upon completion, the orange precipitate was collected by vacuum filtration and washed with chilled MeOH yielding an orange solid. The orange solid was recrystallized in hot MeOH, the orange solid was collected by vacuum filtration to yield an orange solid (68.9 mg, 19%): mp 250-253°C TLC R$_f$ 0.22 80% EtOAc in hexanes. $^1$H NMR (600 MHz, d$_6$-DMSO) δ 11.73 (s, 1H), 10.28 (s, 1H), 8.08-8.07 (d, 2H, J=8.4 Hz), 8.03-8.01 (d, 1H, J=15.24 Hz), 7.76-7.75 (d, 2H, J=8.76 Hz), 7.45-7.43 (d, 1H, J=15.3 Hz), 7.41-7.40 (d, 1H, J=2.34 Hz), 7.30-7.29 (d, 1H, J=8.64 Hz), 6.84-6.82 (dd, 1H, $J_1$=2.4 Hz, $J_2$=2.4 Hz), 3.87 (s, 3H), 2.57 (s, 3H), 2.10 (s, 3H); $^{13}$C NMR (150 MHz, d$_6$-DMSO) δ 187.7, 169.3, 155.4, 144.5, 143.4, 137.8, 133.7, 131.4, 129.7, 118.8, 114.1, 111.2, 103.5, 56.0, 24.7, 12.6. Elemental analysis calcd for C$_{21}$H$_{20}$N$_2$O$_3$ H$_2$O: C, 68.84; H, 6.05; N, 7.65; Found: C, 68.60; H, 6.17; N, 7.51.
Tert-butoxycarbonyl-4-acetylanaline (32)

4-Aminoacetophene (502.6 mg, 3.7 mmol) was dissolved in THF (8 mL). Di-tert-butyl-dicarbonate (1.21 g, 5.5 mmol) was added and refluxed overnight. Upon completion the reaction mixture was concentrated in vacuo to produce a white solid which was partitioned between DCM (25 mL) and brine (25 mL). The organic layer was dried over anhydrous magnesium sulfate. The filtrate was concentrated in vacuo to produce a white solid which was column purified with a gradient of 20-80% EtOAc in hexanes and then repurified with a gradient of 0-20% EtOAc in hexanes. The pure fractions were collected and concentrated in vacuo to yield a white solid (722.8 mg, 82%): mp 144-147 °C TLC Rf 0.26 20% EtOAc in hexanes. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta 7.92-7.90 \text{ (d, 2H, } J=7.5 \text{ Hz), 7.45-7.44 \text{ (d, 2H, } J=7.9 \text{ Hz), 6.69 (s, 1H), 2.56 (s, 3H), 1.53 (s, 9H); } ^{13}\text{C NMR (150 MHz CDCl}_3\text{) 197.0, 152.3, 143.0, 132.0, 130.0, 129.1, 128.2, 117.5, 83.4, 81.5, 28.4, 28.0, 26.5.}\)

3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(N-boc-aminophenyl)-2-propen-1-one (4)

5-Methoxy-2-methylindole-3-carboxyaldehyde (250 mg, 1.3 mmol) was dissolved in MeOH (12 mL). Piperidine (112 mg, 1.3 mmol) and tert-butoxycarbonyl-4-acetylanaline (466 mg, 2.0 mmol) were added and the mixture was allowed to reflux for 12 h. An additional tert-butoxycarbonyl-4-acetylanaline (156 mg, 0.66 mmol) was added and refluxed for 12 h. An orange precipitate slowly begun to form. Upon completion, the orange precipitate was collected by vacuum filtration, washed with chilled MeOH and dried in vacuo yielding an orange solid (205 mg, 38%): mp 229-230 °C TLC Rf 0.63 80%
EtOAc in hexanes. $^1$H NMR (600 MHz, d$_6$-DMSO) δ 11.72 (s, 1H), 9.77 (s, 1H), 8.05-8.03 (d, 2H, $J$=9.48 Hz), 8.02-7.99 (d, 1H, $J$=15.3 Hz), 7.64-7.63 (d, 2H, $J$=8.82 Hz), 7.45-7.42 (d, 1H, $J$=15.3 Hz), 7.40-7.39 (d, 1H, $J$=2.34 Hz), 7.30-7.29 (d, 1H, $J$=8.7 Hz), 6.83-6.82 (dd, 1H, $J_1$=2.34 Hz, $J_2$=2.34 Hz), 3.87 (s, 3H), 2.57 (s, 1H), 1.51 (s, 9H); $^{13}$C NMR (150 MHz, d$_6$-DMSO) δ 187.6, 155.4, 153.0, 144.4, 143.9, 137.6, 132.9, 131.3, 129.7, 127.1, 117.8, 114.1, 112.6, 111.2, 109.5, 103.5, 80.1, 55.96, 28.5, 12.6. Elemental analysis calcd for C$_{24}$H$_{26}$N$_2$O$_4$: C, 70.92; H, 6.45; N, 6.89; Found: C, 70.94; H, 6.37; N, 6.94.

3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(aminophenyl)-2-propen-1-one (5)

3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(N-boc-aminophenyl)-2-propen-1-one (4, 44 mg, 0.11 mmol) was dissolved in DCM (4 mL) then TFA (1 mL) and stirred at rt for 1 h. The reaction mixture was then quenched with NEt$_3$ (~1 mL) dropwise until basic. The reaction mixture was then column purified with a gradient of 50-80% EtOAc in hexanes. The pure fractions were collected and concentrated in vacuo to yield a red-orange solid (15 mg, 44%). TLC R$_f$ 0.43 80% EtOAc in hexanes.$^1$H NMR (600 MHz, d$_6$-DMSO) δ 11.61 (s, 1H), 7.93-7.91 (d, 1H, $J$=15.36 Hz), 7.86-7.85 (d, 2H, $J$=8.7 Hz), 7.41-7.39 (d, 1H, $J$=15.3 Hz), 7.36-7.35 (d, 1H, $J$=2.28 Hz), 7.29-7.28 (d, 1H, $J$=8.64 Hz), 6.82-6.81 (dd, 1H, $J_1$=2.4 Hz, $J_2$=2.4 Hz), 6.65-6.61 (m, 2H), 6.55-6.53 (m, 1H), 6.01 (s, 2H), 3.86 (s, 3H), 2.55 (s, 3H); $^{13}$C NMR (150 MHz, d$_6$-DMSO) δ 183.56, 155.17, 153.80, 153.75, 153.58, 143.25, 135.91, 121.91, 130.83, 127.14, 114.75, 113.24, 110.92, 109.36, 103.32, 55.94, 13.28, 12.63. Elemental analysis calcd for C$_{19}$H$_{18}$N$_2$O$_2$: C, 67.85; H, 4.80; N, 8.33; Found: C, 67.78; H, 4.92; N, 8.35.
3-(5-Methoxy-2-methyl-1H-indol-3-yl)-1-(nitrophenyl)-2-propen-1-one (6)

5-Methoxy-2-methylindole-3-carboxyaldehyde (200 mg, 1.1 mmol) was dissolved in MeOH (12 mL). Piperidine (90 mg, 1.1 mmol) and 4-nitrophenone (262 mg, 1.6 mmol) were added and the mixture was allowed to reflux for 12 h. A red precipitate slowly begun to form. Upon completion, the red precipitate was collected by vacuum filtration, washed with chilled MeOH and dried in vacuo yielding a red solid (328 mg, 92%): mp 250-251 °C TLC Rf 0.52 80% EtOAc in hexanes. 1H NMR (600 MHz, d6-DMSO) δ 11.91 (s, 1H), 8.38-8.36 (d, 2 H, J=11.04 Hz) 8.31-8.29 (d, 2 H, J=11.16 Hz), 8.11-8.09 (d, 1 H, J=15.18 Hz), 7.44-7.42 (d, 1 H, J=2.28 Hz), 7.42-7.40 (d, 1 H, J=15.18 Hz), 7.33-7.31 (d, 1 H, J=8.7 Hz), 6.86-6.84 (dd, 1 H, J1= 2.34 Hz, J2=2.34 Hz), 3.87 (s, 3H), 2.59 (s, 3H) ; 13C NMR (150 MHz, d6-DMSO) δ 155.7, 149.7, 146.3, 144.5, 140.0, 131.5, 129.8, 127.1, 124.3, 113.6, 112.8, 111.3, 109.8, 104.1, 56.1, 12.6. Elemental analysis calcld for C19H16N2O4: C, 67.85; H, 4.80; N, 8.33; Found: C, 67.78; H, 4.92; N, 8.35.
References


8. Nair, Devatha P.; Podgorski, Maciej; Chatani, Shunsuke; Gong, Tao; Xi, Weisian; Fenoli, Christopher R.; Bowman, Christopher N. The Thiol-Michael Addition Click Reaction: A Powerful and Widely Used Tool in Materials Chemistry. *Chemistry of Materials*.


11. Schirmer, Andreas; Kennedy, Jonathan; Muri, Sumati; Reid, Ralph; Santi, Daniel V. Targeted covalent inactivation of protein kinases by resorcylic acid lactone polyketides. *PNAS* 2006, 103(11); 4234-4239.


Appendix A

Biological Activity Data

SRB Data. JO-1-49 (7), JO-1-51 (8), JO-1-57 (9), JO-1-58 (10)
Phase Pictures
Appendix B

NMR Spectra for Synthesized Compounds