SYNTHESIS AND BIOLOGICAL ACTIVITY OF *N-trans*-COUMAROYLTYRAMINE DERIVATIVES

BY

MISS PAILIN PETHTHAI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE (CHEMISTRY)

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE AND TECHNOLOGY

THAMMASAT UNIVERSITY

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was approved as partial fulfillment of the requirements for
the degree of Master of Science (chemistry)

on September 18, 2020

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ABSTRACT

trans-N-coumaroyltyramine, a natural compound isolated from *Nelumbo nucifera*, shows potent biological activity as an antioxidant, and has anti-obesity capacity by inhibiting pancreatic lipase and adipocyte differentiation. We are interested in the synthesis of *trans-N*-coumaroyltyramine derivatives, an amide, aimed to study the derivatives and the structure activity relationship. In this work, we optimized the procedure to construct the *trans-N*-coumaroyltyramine derivatives using direct thermal reaction, coupling reagent method and microwave irradiation, and reported a simple and efficient; two-step, one-pot synthetic method for the direct conversion of aromatic aldehydes to cinnamate amides and did studies with and without purification of the acids synthesized from the first step. The 24 synthesized compounds were study the biological activities, results of antioxidant activity showed that 4-hydroxy-*N*-[2-(4-hydroxyphenyl) ethyl] -3-methoxy-benzamide has the greatest IC_{50} value of 38 µg/mL when gallic acid (IC_{50} = 3.68 µg/mL) as positive control. In addition, *N*-Benzyl-4-methoxycinnamamide and *N*-Benzyl-4-fluorocinnamamide can inhibited feeding and insecticide the larvae of common cutworm. Moreover, and *N*-benzyl-3-(4-fluorophenyl)prop-2-enamide can delay of plant growth.

Keywords: *trans-N*-coumaroyltyramine derivatives; Amide synthesis; Antioxidant activity

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Miss Pailin Peththai

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
AFI	Antifeedant Index
$CDCl_3$	Deuterated chloroform
DIC	1,3-Diisopropylcarbodiimide
DCC	Dicyclohaxylcarboiimide
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
d	Doublet
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOAc	Ethyl acetate
eq	Equivalent
FTIR	Fourier-transform infrared spectroscopy
g	Gram
HOBt	Hydroxybenzotriazole
IC_{50}	Half maximal inhibitory concentration
M	Molar
MHz	Megahertz
m	Multiplet
mg	Milligram
mL	Milliliter
mmol	Millimole
min	Minute
mp	Melting point
MW	Molecular weight
MWI	Microwave Irradiation

NMR	Nuclear magnetic resonance spectroscopy
nm	nanometer
p-NPL	*p*-Nitrophenyl laurate
ppm	Parts per million
rt	Room temperature
TLC	Thin-layer chromatography
t	Triplet
s	Singlet
μg	Microgram
μM	Micromolar
μL	Microliter
R^2	Coefficient of determination
°C	Degree Celsius

CHAPTER 1
INTRODUCTION

1.1 Thesis motivation

Nowadays, obesity is a very common problem. It is a condition in which the body accumulates excess fat which is a risk factor or cause various diseases affecting health such as heart disease, diabetes, certain types of cancer, and osteoarthritis that may cause death. The main cause of obesity is eating more food than necessary for the body and the lack of regular exercise. There are also other factors such as heredity, age, sex, medication, or psychiatric illness, etc. that contribute of obesity. Obesity begins with fats received from food. Fat is absorbed in the small intestine after being hydrolyzed by pancreatic lipase. Excessive energy can lead to excess fat accumulation in adipocytes and lead to obesity.

In medicine, there are two ways to stop obesity: blocking fat absorption by inhibiting lipase produced by the pancreas and inhibiting fat accumulation by interfering with differentiation of adipocytes tissue. Previous studies have shown that *Nelumbo nucifera* Gaertn (*Nymphaeaceae*), an aquatic plant that has spread throughout East Asia, has antioxidants, anti-HIV, diabetes prevention and obesity prevention as well.

1.2 Plant research: *Nelumbo nucifera* Gaertn (*Nymphaeaceae*)

Nelumbo nucifera Gaertn belonging to the *Nymphaeaceae* family, commonly called lotus, is an aquatic plant that has been distributed mostly in eastern Asia. All parts of the lotus plant, including leaves, flowers, stamens, embryos, and rhizomes are edible and have been used as food for a long time (Figure 1.1). Also, some parts have been used as traditional medicines due to pharmacologic and physiologic activities as antifebrile, sedative, antibacterial, antidiarrheal, and hemostatic agents.[1]

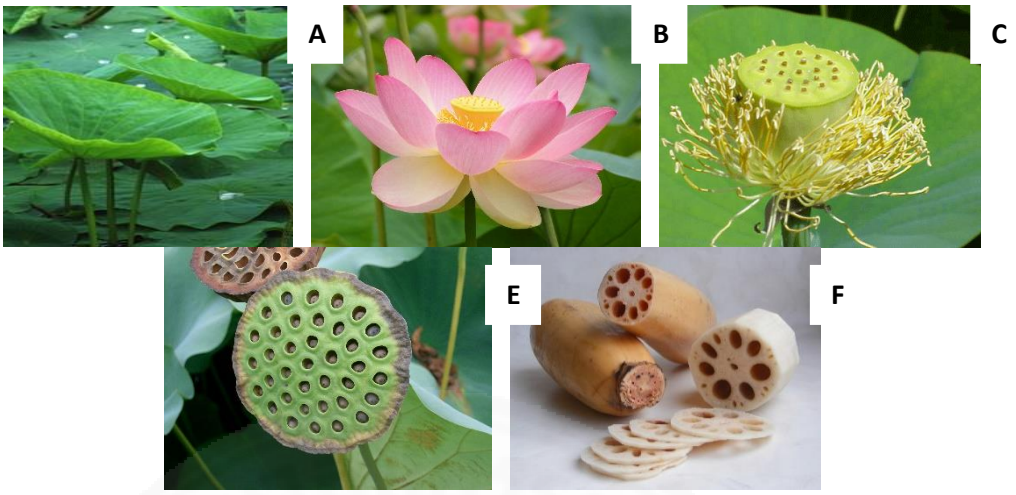


Figure 1.1 *Nelumbo nucifera* Gaertn (*Nymphaeaceae*): (A) leaves, (B) flowers, (C) stamens, (E) embryos, and (F) rhizomes. [1]

Previous studies revealed the pharmacological effects of lotus leaves in, for example, hepatoprotective,[2] antioxidant activity,[3] antidiarrheal,[4] antiviral,[5] immunomodulatory,[6] and anti-obesity[7] effects. The beneficial effects of lotus leaves on obesity have been extensively investigated. Lotus leaf extract decreases obesity by reducing fat accumulation and by activating lipolysis and hypolipidemic in adipocytes and in high-fat animals.[7,8,9] From the literature, lotus leaf extract contains several flavonoids, including (*E*)-3-hydroxymegastigm-7-en-9-one (**1**), (-)-boscialin (**2**). Some alkaloids, such as *trans-N*-coumaroyltyramine (**3**), *cis-N*-coumaroyltyramine (**4**), *trans-N*-feruloyltyramine (**5**), and *cis-N*-feruloyltyramine (**6**) (Figure 1.2) can also be present.[10]

We are interested in coumaroyl tyramine derivatives found in lotus leaves especially compounds **3** and **5** because they are significantly active toward the inhibition of the lipase enzyme produced by the pancreas. The inhibition of this enzyme could lead to weight loss. In addition, the related derivatives as *trans-N*-coumaroyltyramine (**3**), *cis-N*-coumaroyltyramine (**4**), *trans-N*-feruloyltyramine (**5**) and *cis-N*-feruloyltyramine (**6**) show many biological activities including anti-inflammatory,[11] antioxidant activity,[11] acetylcholinesterase enzyme inhibition,[11] anti-adipogenic,[10] and pancreatic lipase[10] activities.

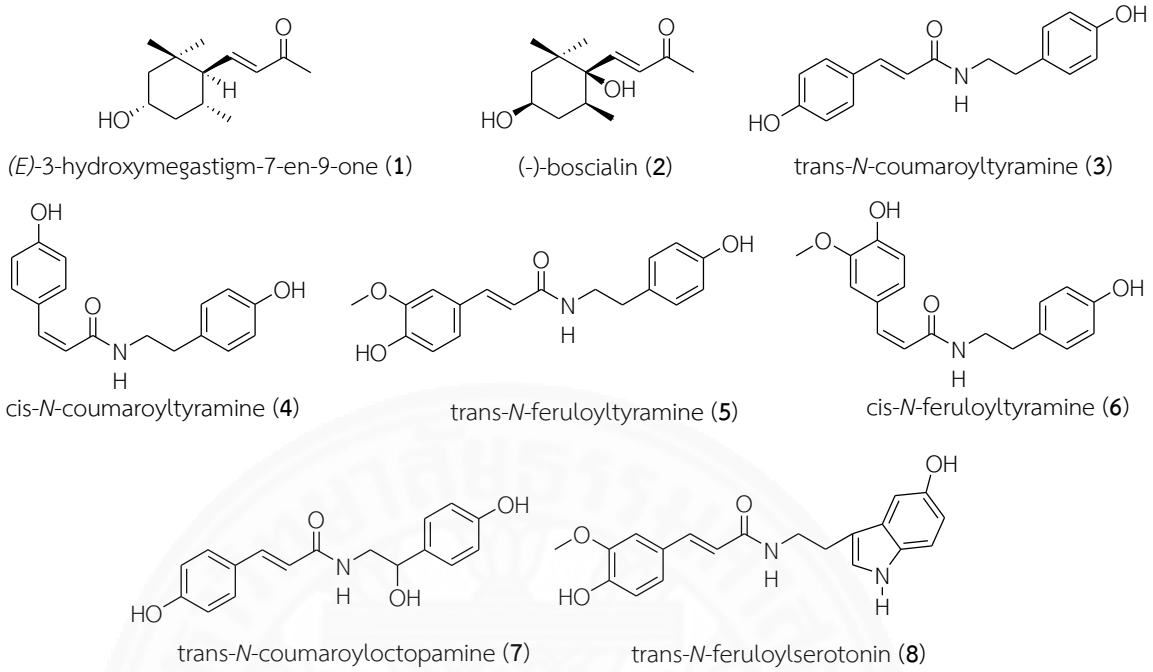


Figure 1.2 Example of flavonoid and alkaloid structures in lotus leaves.

Therefore, this research aims to synthesize *trans-N*-coumaroyltyramine derivatives or called phenylpropanoid amides in which the amide bond was made from cinnamic acid derivatives and amines. The synthesized compounds are then subjected to evaluation of antioxidant activities for the initial information before testing pancreatic lipase inhibition. For the long term, we expect to provide the structure activity relationship of the derivatives toward antioxidant activity and pancreatic lipase inhibition.

1.3 Research objectives

1.3.1 To find a simple and efficient synthetic method to synthesize *trans-N*-coumaroyltyramine derivatives.

1.3.2 To study the biological activities of the synthesized compounds such as phytotoxicity, insecticidal activity, antifeedant activity, antioxidant activity, and antipancreatic lipase activity.

1.4 Expected outcomes

1.4.1 Obtain a simple method for the synthesis of *trans-N*-coumaroyltyramine derivatives.

1.4.2 Obtain a variety of *trans-N*-coumaroyltyramine derivatives.

1.4.3 Study the structure activity relationship (SAR) from the synthesized *trans-N*-coumaroyltyramine derivatives

CHAPTER 2
LITERATURE REVIEWS

2.1 Introduction

Nelumbo nucifera Gaertn (*Nymphaeaceae*), typically called lotus, is a freshwater plant that is widely distributed throughout East Asia. People treat the elements of the lotus plant, including fruits, seeds, roots, and leaves as food and drink for centuries. In addition, the lotus elements are long known to be used as folk medicine, for example, as antidepressants, sedatives, antibiotics, and as a cure for diarrhea and hemorrhoids.[1] Previous research has revealed the pharmacological effects of lotus leave as anti-oxidants,[12] anti-HIV,[13] anti-diabetes,[14]and anti-obesity.[7]

There is extensive investigation into the ability of lotus leaves to inhibit obesity. The lotus leaf extracts help to prevent obesity by reducing fat accumulation, stimulating fat, and reducing fat in apical tissue, also known as fatty tissue. When the lotus leaf was dissolved in ethyl acetate, the soluble fraction in ethyl acetate produced a change in fatty tissue and inhibited the lipase from the pancreas. The phytochemicals were examined and the total of 33 compounds were extracted from lotus leaves.[10]

The isolated compounds were identified by the analysis of spectroscopic data and comparison with literature values, as (*E*)-3-hydroxymegastigm-7-en-9-one (**1**), (-)-boscialin (**2**),2 (+)-dehydrovomifoliol (**9**), vomifoliol (**10**), 3-oxo-retro-α-ionol I (**11**), byzantionoside A (**12**), 5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**13**), annuionone D (**14**), icariside B2 (**15**), grasshopper ketone (**16**), 3S,5R-dihydroxy-6S,7-megastigmadien-9-one (**17**), (+)-epiloliolide (**18**), (6R,6aR)-roemerine-Nβ-oxide (**19**), liriodenine (**20**), pronuciferine (**21**), oleracein E (**22**), *trans-N*-coumaroyltyramine (**3**), *cis-N*-coumaroyltyramine (**4**), *trans-N*-feruloyltyramine (**5**), *cis-N*-feruloyltyramine (**6**), quercetin (**23**), kaempherol (**24**), luteolin (**25**), quercetin 3-O-glucopyranoside (**26**), kaempherol 3-O-glucopyranoside (**27**), chrysoeriol 7-O-glucopyranoside (**28**), taxifolin (**29**), epitaxifolin (**30**), 5,7,3',5'-tetrahydroxyflavanone (**31**), (-)-catechin (**32**), and elephantorrhizol (**33**).

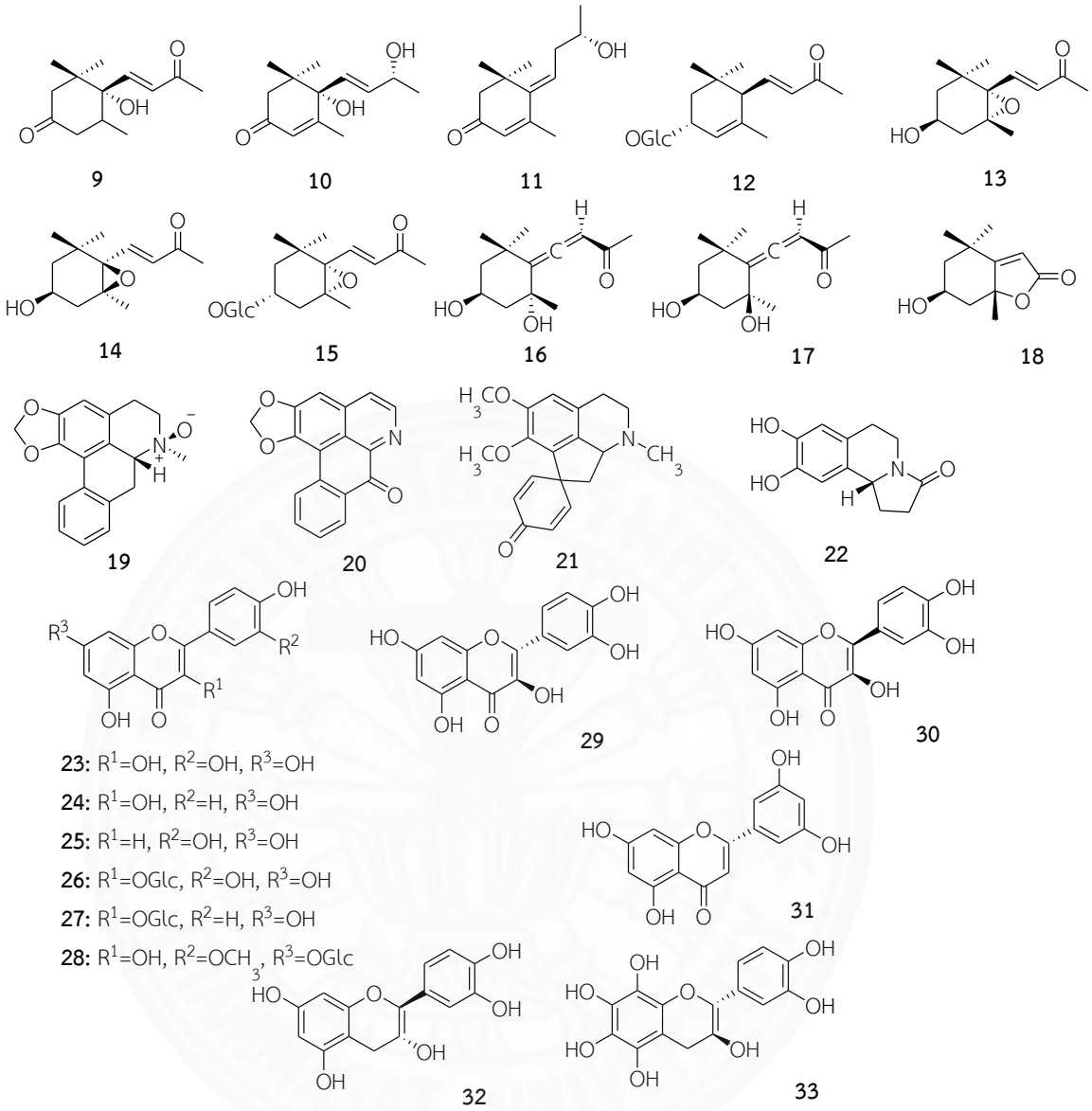


Figure 2.1 Structures of compounds from the leaves of *Nelumbo nucifera.*

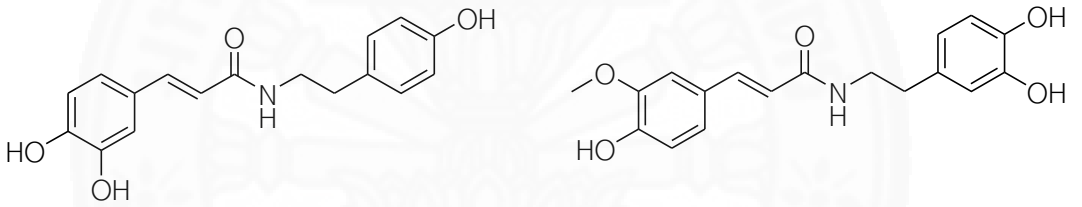
The leaf extract from *N. nucifera* is composed of 33 compounds as mentioned above. The biological activity results suggest that coumaroyltyramines (**3** and **4**) and feruloyltyramine (**5** and **6**) are the most significant inhibitor toward both lipid differentiation and pancreatic lipase. Based on the simplicity of chemical structure, we choose to investigate the structure activity relationship using *trans-N*-coumaroyltyramine **3** as a lead structure.

2.2 Biological activities of coumaroyltyramine derivatives

Coumaroyltyramine derivatives exhibits diverse biological activities such as anti-inflammatory,[11] antioxidant activity,[11] acetylcholinesterase enzyme inhibition,[11] anti-adipogenic,[10] and pancreatic lipase[10] activities.

2.2.1 Antioxidant activity

Al-Taweel *et al*., 2012[11] demonstrated that *trans-N*-feruloyltyramine (**5**) *trans-N*-caffeoyltyramine (**34**) are effective for 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical activity with IC_{50} of **5** and **34** equal 33.2 and 26.2 μM. In addition, Batsukh Odonbayar *et al*., 2016[15] demonstrated that the phenylpropanoid amides (**34**) also had DPPH radical scavenging activity with the IC_{50} of 30.7 μM.



trans-N-caffeoyltyramine (**34**) *trans-N*-feruloyldopamine (**35**)

Figure 2.2 The chemical structures of *trans-N*-caffeoyltyramine (**34**) and trans-*N*-coumaroyldopamine (**35**).

2.2.2 Acetylcholinesterase enzyme inhibition

An acetylcholinesterase inhibitor (often abbreviated AChEI) or anti-cholinesterase is a chemical or a drug that inhibits the acetylcholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine.

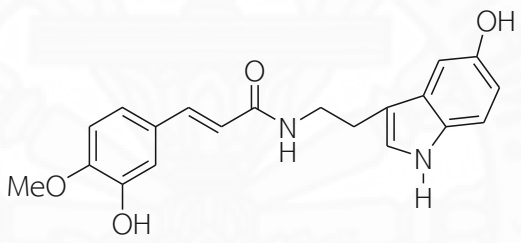
Al-Taweel *et al*., 2012[11] demonstrated that trans-*N*-coumaroyltyramine (**3**) and trans-*N*-feruloyltyramine (**5**) show moderate acetylcholinestrease enzyme inhibition activity. In addition, Kim, and Lee., 2003[16] reported that *N-p*-coumaroyl tyramine (**3**), show inhibitory activity on acetylcholinesterase (ACHE) with the IC_{50} value 34.5 μg/mL (122 μM).

2.2.3 Anti-alpha glucosidase activities

Y.H. Song *et al*., 2016[17] demonstrated that *trans*- *N*-coumaroyltyramine (**3**) showed significant inhibition of **α**-glucosidase (IC_{50} = 0.42 mM). Moreover, Silva and Borges *et al*., 2017[18] reported *trans N*-cumaroyloctopamine (**7**) and *trans*- *N*-cumaroyltyramine (**3**) are examples of strong **α**-glucosidase inhibitors.

2.2.4 Tyrosinase inhibition

Yang *et al*., 2018[19] demonstrated that *trans-N*-coumaroyltyramine (**3**) and *cis-N*-coumaroyltyramine (**4**) showed tyrosinase inhibition with IC_{50} values of 40.6 and 36.4 **μ**M. Takahashi *et al*., 2010[20] also reported *trans-N*-isoferuloylserotonin (**36**) exhibited significant tyrosinase inhibitory activity (IC_{50} = 5.4 ± 3.6 **μ**M).

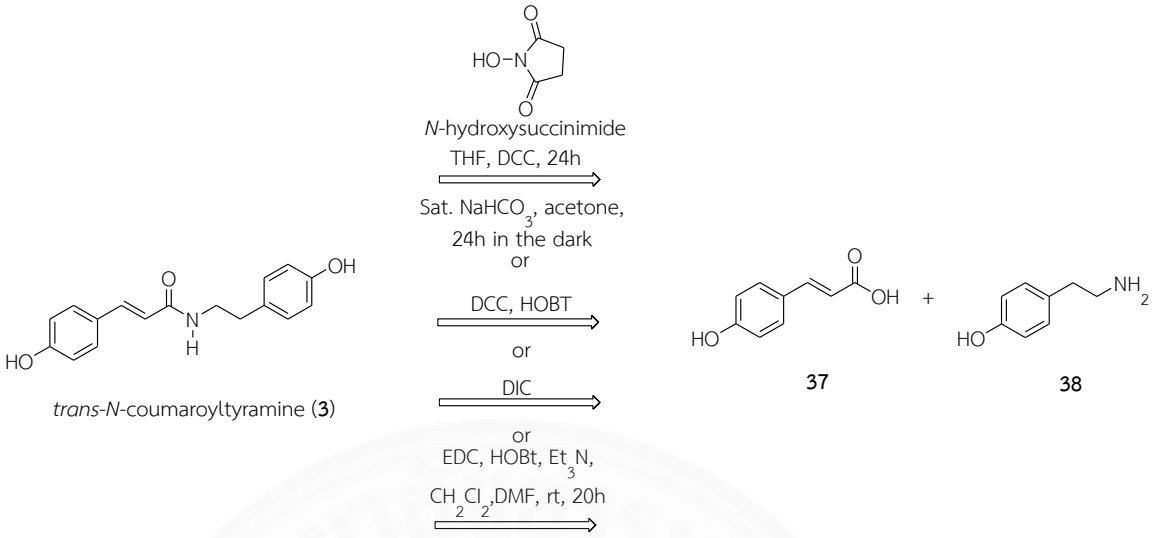


trans-N-isoferuloylserotonin(**36**)

Figure 2.3 The chemical structures of *trans-N*-isoferuloylserotonin (**36**).

2.3 Synthesis of *trans-N*-coumaroyltyramines (**3**)

trans-N-coumaroyltyramine (**3**) is a natural compound isolated from several plants such as *Abelmoschus sagittifolius, Celtischinensis, Lycium chinenese, Atraphaxis frutescens, and Nelumbo nucifera*. The compound shows potent inhibitory activity as an anti-obesity agent by inhibiting pancreatic lipase and adipocyte differentiation. From the literature survey, *trans-N*-coumaroyltyramine (**3**) could be synthesized from an amidation reaction between *p*-coumaric acid and tyramine using coupling reagents including 1,3-diisopropylcarbodiimide (DIC),[21] 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in the presence of hydroxybenzotriazole (HOBt)[22], DCC/HOBt[23] and *N*-hydroxysuccinimide[24]. The synthesis scheme is shown in Scheme 2.1.



Scheme 2.1 Retro synthesis of *trans-N*-coumaroyltyramines (**3**)

2.4 Synthesis of *trans-N*-coumaroyltyramine derivatives via amide bond formation

Amides are a common functional group found in several small or complex synthetic or natural molecules including synthetic polymers, peptides, pharmaceutical agents, and many others. The presence of an amide functional group is featured in several commercial drugs, including penicillin G (a well-known antibiotic drug), lidocaine (an active ingredient in insecticide) and sultopride (an antipsychotic drug). The structures are shown in Figure 2.4.

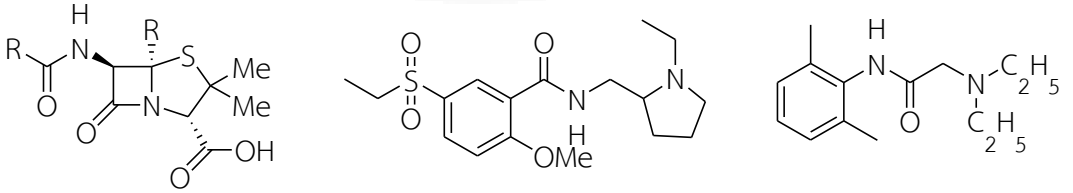


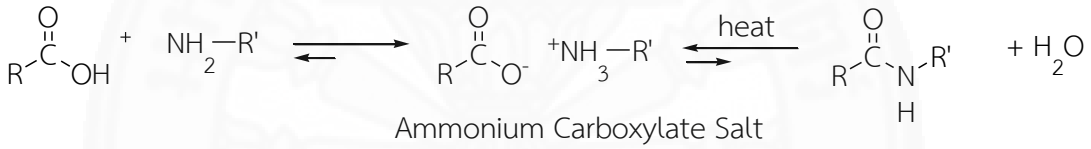
Figure 2.4 Examples of drugs containing an amide bond.

Typically, the synthesis of amides can be categorized into three synthetic pathways: thermal direct condensation, activation of acids via coupling reagents, and

microwave-assisted amide formation. The other alternative methods include Schotten-Baumann reaction, Beckmann rearrangement, Willgerodt reaction, Ugi reaction, Chapman rearrangement, and dehydrogenative acylation. In this review, we only focused on the common three methods.

2.4.1 Thermal direct condensation

Amide bond formation between an acid and an amine are basically condensation reaction. This is an equilibrium reaction upon mixing an amine with a carboxylic acid, in which an acid–base reaction occurs first to form an ammonium carboxylate salt. The intermediate salt is so stable that need to be heat at least 100°C to push the reaction forward. In other words, the amide bond formation must fight against adverse thermodynamics as the equilibrium shown in Scheme 2.2.

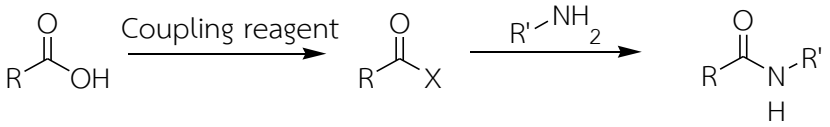


Scheme 2.2 Thermal direct condensation.

As above mentioned, the direct condensation of the salt is achieved at high temperatures, which is often incompatible in the presence of other functional groups in the chemical structure. As a result, acid activation is commonly required to promote the coupling reaction with an amine to avoid the decomposition of the starting material. The development of safe and efficient processes for acid activation and amine condensation are still one of the important topics, especially on an industrial scale.

2.4.2 Acid activation via coupling reagents

The conversion of carboxylic acids to amides is one of the important transformations in organic synthesis. In general, the conversion of carboxylic acids to amides requires an activation of the carboxyl group.

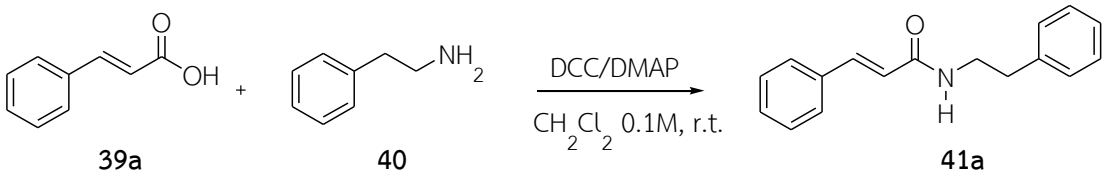


Scheme 2.3 Amide coupling method.

This can be achieved by conversion of the carboxyl group to a more reactive group such as acyl halide, mixed anhydride, acyl azide, and active ester. This activation step could be achieved through in situ–activation with coupling reagents. Those coupling reagents, for example, are *N,N*-dicyclohexyl carbodiimide (DCC), $TiCl_4$, activated phosphate, $Sn[N(TMS)_2]_2$, *N*-halosuccinimide/PPh_3, trichloro acetonitrile/PPh_3, tert-butyl-3-(3,4 dihydrobenzotriazine-4-on)yl carbonate (Boc-Odhbt), $ArB(OH)_2$, Lawesson's reagent, $(R_2N)_2Mg$, SO_2ClF, chlorosulfonyl isocyanate, and 2-mercaptopyridone-1-oxide based uranium salts. Among several existences of coupling reagents reported, we are interested in three common coupling reagents: triazine-based compounds[25], carbonic anhydrides or active esters[26], and boric acid derivatives[27].

2.4.2.1 Coupling via Activated Ester

The Steglich esterification is a long-known coupling reaction of acids and alcohols using dicyclohexylcarbodiimide as a coupling reagent mixed with 4-dimethylaminopyridine as a catalyst. The reaction was first described by Wolfgang Steglich in 1978.[26] The use of DCC, or *N, N′*-dicyclohexylcarbodiimide, for the formation of peptide and other amide bonds was first reported by Sheehan and Hess in 1955.[28] Many carbodiimides have been investigated as coupling reagents such as DCC, DIC (*N, N′*-diisopropylcarbodiimide), and EDC (1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride, but only a few are routinely used on large scale based on availability, cost, isolation, and environmental considerations. Later, this reaction has been adapted to peptide synthesis practically in small scale lab by means of using DCC (dicyclohexylcarbodiimide) in the presence of 1-hydroxybenzotriazole (HOBt). [29,30]

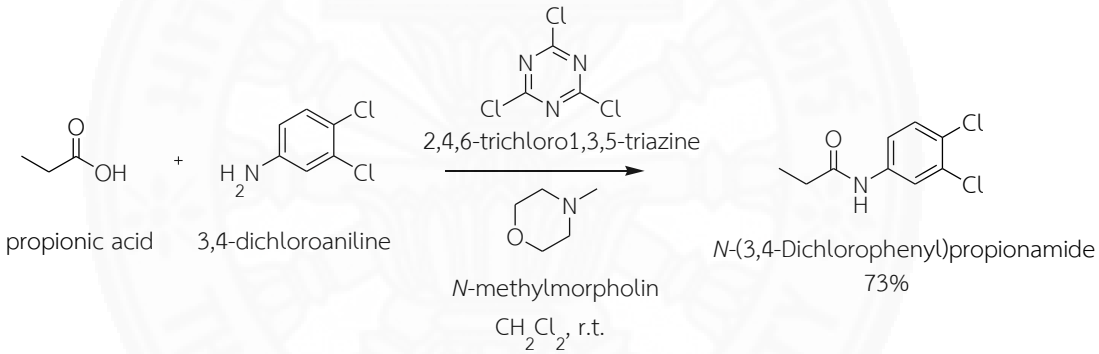


39a **40** **41a**

Scheme 2.4 Synthesis amide by use of DCC, or *N, N´*-dicyclohexylcarbodiimide as a amide coupling reagent.

2.4.2.2 Triazine-Based Coupling Reagents

Rayle and Fellmeth., 1999[25] described that cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) can be used to convert carboxylic acid to acyl chloride by reacting with N-methylmorpholine (NMM) to control pH conditions throughout the reaction.



propionic acid 3,4-dichloroaniline *N*-(3,4-Dichlorophenyl)propionamide 73%

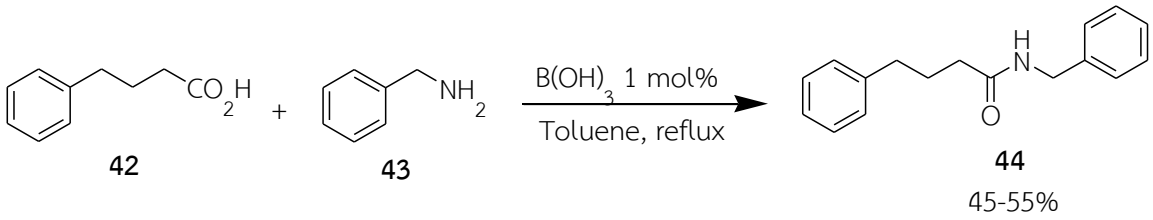
Scheme 2.5 Cyanuric chloride as an acid-activating reagent.

Cyanuric chloride is a coupling reagent suitable for a large-scale amide synthesis. The advantage of using this type of coupling reagent is to use small quantities resulting in byproduct that is easy to be removed by filtration or washing with a base.

2.4.2.3 Coupling via Boron Species

Boric acid catalyzed carboxylic amide formation directly from carboxylic acids and amines. In comparison to the direct thermal condensation, boric acid and the derivatives prove to form a complex with hydroxyl functionality and provide higher catalytic activity with high yield and lower in temperature. Tang *et al*., 2005[27] exemplified the synthesis of amide as shown in Scheme 2.6 This reaction has attracted

considerable attention because it has emerged as a viable alternative route to the indirect methods of preparing amides and it is inexpensive.

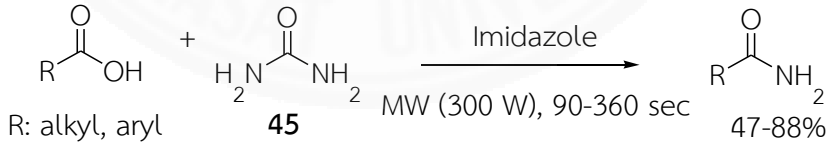


Scheme 2.6 The synthesis of amide using boric acid as catalyst.

2.4.3 Microwave-assisted amide synthesis

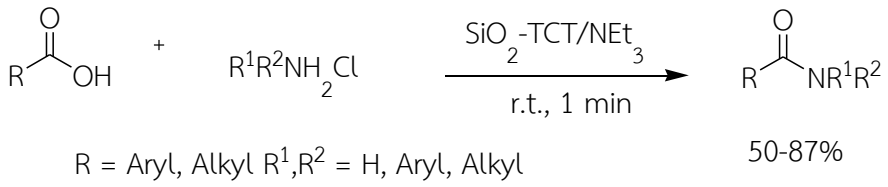
In recent years, microwave irradiation has been increasingly used in organic reactions due to the short reaction time and high yield compared to conventional heating. Several organic reactions have been reported to be facilitated by microwave irradiation such as Diels-Alder, ene, Claisen reactions, Knoevenagel condensation, Fischer cyclization, Hantzsch synthesis, Michael addition, Heck reaction, oxidation, reduction, hydrolysis of ester, amide formation and others. [31,32]

For the amide synthesis, Khalafi-Nezhad *et al.*, 2003[33] demonstrated the very simple and efficient solvent-free procedure in the presence of imidazole under microwave irradiation. Various aliphatic and aromatic primary amides were prepared in good yields by this direct amidation method (Scheme 2.7).



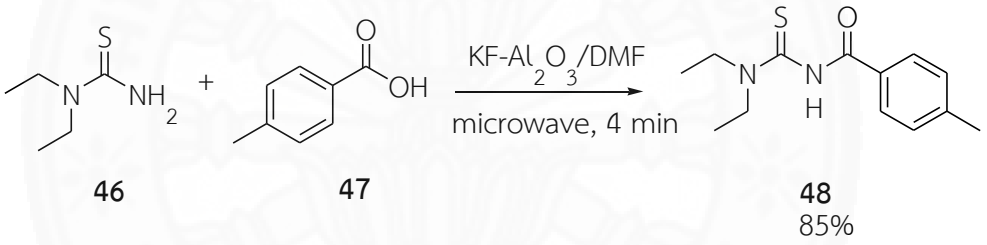
Scheme 2.7 The synthesis of primary amides by direct reaction of carboxylic acid and urea in the presence of imidazole under microwave irradiation.

Next, Khalafi-Nezhad *et al.*, 2017[34] reported that an efficient, rapid, and simple method for the direct conversion of carboxylic acids to primary, secondary, tertiary alkyl, and aromatic amides in the presence of the corresponding ammonium salts, silica-mediated 2,4,6-trichloro-1,3,5-triazine (TCT), and triethylamine under solvent-free conditions at room temperature with moderate to excellent yields (Scheme 2.8).



Scheme 2.8 The direct conversion of carboxylic acids to primary, secondary, tertiary alkyl, and aromatic amides.

In addition, in 2000 H. Mhrquez *et al*.[35] discovered that amide formation in a compound (**50**) could be synthesized using alumina impregnated with potassium fluoride in the presence of catalytic amounts of *N,N*-dimethylformamide under microwave irradiation using an unmodified domestic microwave oven. The yields are noticeably improved in comparison with classical methods.



Scheme 2.9. The synthesis was carried out in the presence of alumina impregnated with potassium fluoride and catalytic amounts of *N, N*-dimethylformamide.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Research scope

The study aims to find a simple and efficient synthetic method for the synthesis of phenylpropanoid amides or *p*-coumaroyl tyramine derivatives (Figure 3.1) and evaluate the biological activities such as phytotoxicity, insecticidal activity, antifeedant activity, antioxidant activity, and antipancreatic lipase activity.

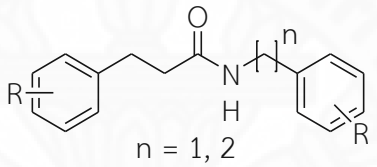


Figure 3.1 Structure of phenylpropanoid amide derivatives.

3.2 Methodology

3.2.1 Chemicals and Reagents

Benzaldehyde (C_7H_6O), *p*-anisaldehyde ($C_8H_8O_2$), and benzylamine, were purchased from Acros Organic. Malonic acid ($C_3H_4O_4$) was purchased from Alfa Aesar. Toluene, xylene, pyridine, piperidine were purchased from CARLO ERBA. All organic solvents were purchased from Italmar (Thailand) and distilled prior to use. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. DI water type I was generated by Elga Labwater. Merck silica gel was used for column chromatography.

3.2.2 Apparatus

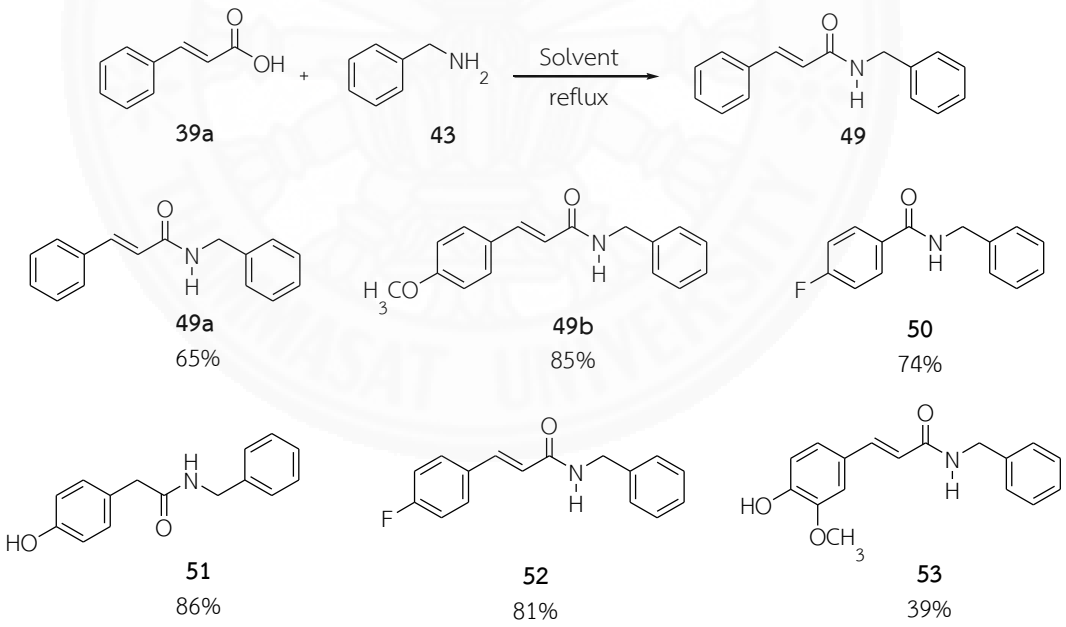
Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254. ^{1}H-NMR spectra were measured by BRUKER-NMR 400MHz spectrometer at 400 MHz using TMS as internal standard and reported as ppm in chloroform-*d* and DMSO-d_6. Mass spectra were measured by Thermo Filingan spectrometer. Melting point were measured by Kruss Optronic KSP1N Automatic Melting Point Apparatus, 30–360 °C

Measuring Range. Microplate-reader were measured by FLUOstar Omega Filter-based multi-mode microplate reader (BMG LABTECH, Weston Parkway, USA) was used for measuring antioxidant, and antipancreatic lipase activity at a wavelength of 510 nm and 405 nm, respectively.

3.3 Synthesis of amide

We investigated the simple methodology reported in the literature to find the practical procedure to synthesize the phenylpropanoid derivatives. To find the optimum conditions, the study of amide synthesis utilized cinnamic acid and *p*-methoxy cinnamic acid as acid starting materials coupling with simple amines including phenyl ethyl amine and benzyl amine. The general procedures are described as follow.

3.3.1 Thermal direct condensation



Scheme 3.1 Direct thermal reaction of cinnamate amide.

General procedure: A mixture of cinnamic acid (**39a**) (0.100 g, 1 mmol), benzylamine (**49a**) (0.109 g, 1.5 mmol), and solvent (3.0 mL) was refluxed for 3-4 hours or until the reaction is complete according to monitoring by TLC. The mixture was

cooled, and the solvent was evaporated by rotary evaporator. The residue was purified by flash column chromatography using hexane and ethyl acetate as eluent.

Compound **49a**: cinnamic acid (651.9 mg, 1.25 mmol) and benzylamine (471.5 mg, 4.4 mmol) were employed to give *N*-benzyl cinnamamide (65%); m.p. 99-102 °C; R_f (30% EtOAc/n-hexane) 0.53; FT-IR (neat) 3297, 1654, 1257, 1051 cm-^{1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.90-7.81 (d, *J* = 15.3 Hz, 1H), 7.58-7.51 (m, 5H), 6.25-6.19 (d, *J* = 22.85 Hz, 1H), 4.51-4.49 (d, *J* = 13.86 Hz, 1H), 3.89 (s, 3H), 3.76-3.73 (d, *J* = 10.36 Hz, 2H)

Compound **49b**: 4-Methoxycinnamic acid (202.8 mg, 1.14 mmol) and benzylamine (496.1 mg, 4.63 mmol) were employed to give *N*-benzyl-4-methoxycinnamamide (85%); m.p. 145-150 °C; R_f (30% EtOAc/n-hexane) 0.38; FT-IR (neat) 3291, 3033, 3000, 3033, 3000, 1606, 1257,1051 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.38-7.19 (m, 4H), 6.83-6.80 (m, 5H), 6.25-6.19 (d, *J* = 22.85 Hz, 1H), 4.51-4.49 (d, *J* = 13.86 Hz, 1H), 3.89 (s, 3H), 3.76-3.73 (d, *J* = 10.36 Hz, 2H)

Compound **50**: 4-Fluorobenzoic acid (500.7 mg, 3.57 mmol) and benzylamine (1530.0 mg, 14.58 mmol) were employed to give *N*-benzyl-4-fluorobenzamide (81%); light yellow solid; m.p. 188-194 °C; R_f (30% EtOAc/n-hexane) 0.60; FT-IR (neat) 3332, 3114, 2975, 1645, 1052 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 9.0 (s, 1H), 7.97-7.93 (m, 2H), 7.32-7.27 (m, 7H), 4.48-4.46 (d, J = 11.3 Hz, 2H).

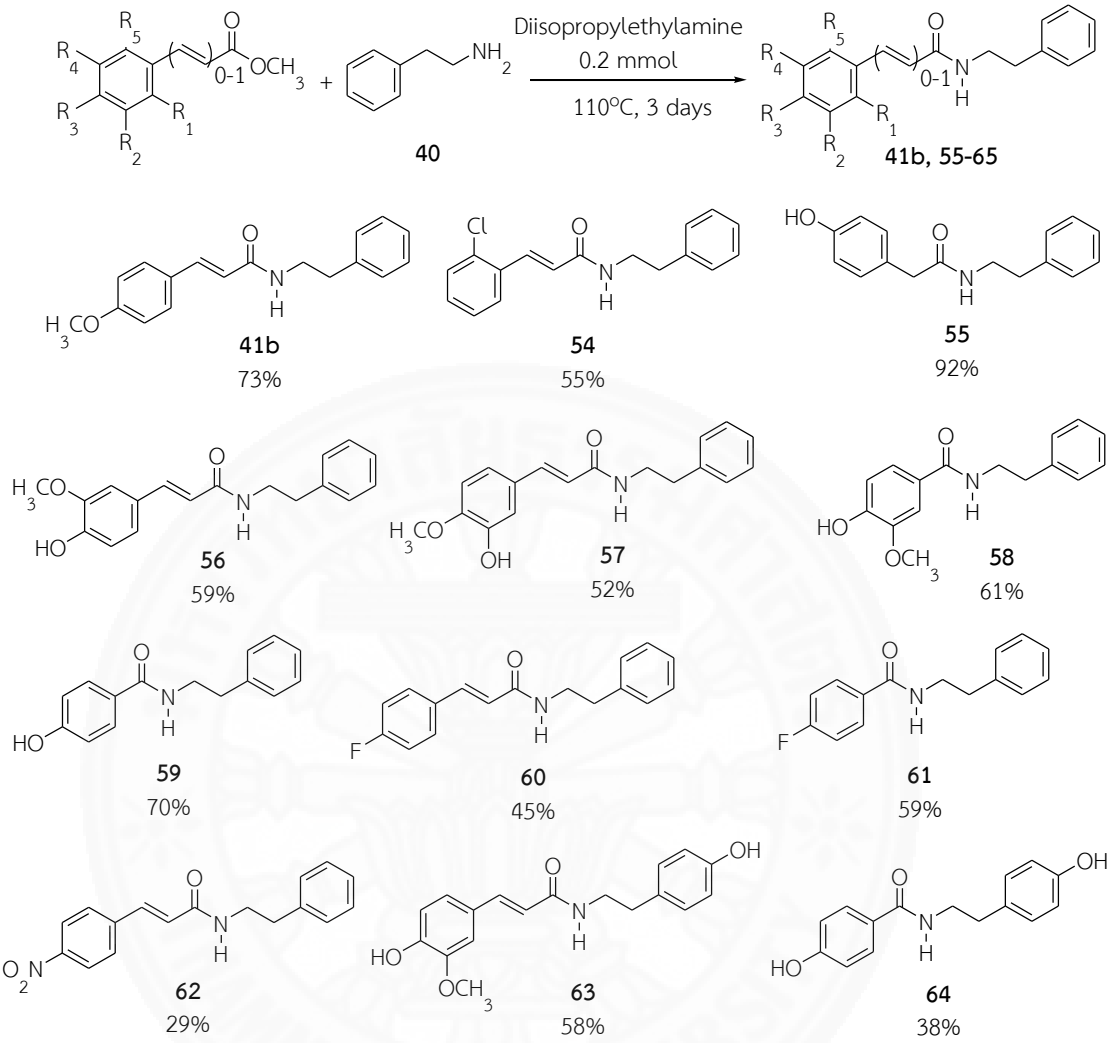
Compound **51**: 4-Hydroxyphenylacetic acid (500.1 mg, 3.29 mmol) and benzylamine (1409 mg, 13.15 mmol) were employed to give *N*-benzyl-2-(4-methoxyphenyl)acetamide (86%); white solid; m.p. 133-143 °C; R_f (30% EtOAc/n-hexane) 0.13; FT-IR (neat) 3400, 3293, 3089, 3088, 3076, 3076, 2937, 2895, 1640 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 9.20 (s, 1H, OH-1), 8.30 (s, 1H), 7.34-7.03 (m, 7H), 6.70-6.60 (d, *J* = 11.9 Hz, 2H), 4.25-4.23 (d, *J* = 11.3 Hz, 2H), 3.7 (s, 2H).

Compound **52:** 4-Fluorocinnamic acid (732.5 mg, 4.41 mmol) and benzylamine (2040 mg, 19.04 mmol) were employed to give *N*-benzyl-3-(4-fluorophenyl)prop-2-

enamide (74%); light yellow solid; m.p. 118-128 °C; R_f (30% EtOAc/n-hexane) 0.37; FT-IR (neat) 3283, 3091, 3060, 2930, 2865, 1659, 1230 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.60-7.70 (br m, 2H), 7.45-7.50 (br m, 1H), 7.40 (s, 2H), 7.37 (s, 1H), 7.33 (s, 2H), 6.35 (br s, 1H), 5.85 (br s ,1H), 4.60 (s, 1H)

Compound **53**: trans-Ferulic acid (500.0 mg, 2.58 mmol) and benzylamine (1103.7 mg, 10.30 mmol) were employed to give (*E*)-*N*-benzyl-3-(4-hydroxy-3-methoxyphenyl) acrylamide (39%); light yellow liquid; R_f (30% EtOAc/n-hexane) 0.78; FT-IR (neat) 3526, 3495, 3092, 3090, 3017, 2968, 1610, 1257 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.04 (s, 1H), 7.03 (d, *J* = 6.01 Hz, 1H), 6.86-6.83 (m, 8H), 6.64 (d, *J* = 12.81 Hz, 1H), 3.78 (s, 3H).

3.3.2 Amide synthesis *via* aminolysis of ester



Scheme 3.2 Amide synthesis *via* aminolysis of ester.

General procedure: In a small vial, a mixture of ester (1.65 mmol, 1 equiv.), phenylethylamine (3.96 mmol, 2.4 equiv.), and diisopropylethylamine 0.2 mmol was heated at 110 °C for 3 days on an oil bath. After completion of the reaction monitored on TLC, the residue was purified by flash column chromatography using hexane and ethyl acetate as the eluent.

Compound **41b**: Methyl 4-methoxycinnamate (306.3 mg, 1.56 mmol), phenylethylamine (453.5 mg, 3.75 mmol), and diisopropylethylamine 0.2 mmol were employed to give (*E*)-3-(4-methoxyphenyl)-*N*-phenethylacrylamide (37%); dark brown

viscous liquid; m.p. 123.4-130.9 °C; R_f (20% EtOAc/n-hexane) 0.15; FT-IR (neat) 3263, 3053, 2934, 1653, 1601, 1256 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 7.69 (d, *J* = 17.5 Hz, 2H), 7.55 (d, *J* = 17.4 Hz, 2H), 7.40 (d, *J* = 21.1 Hz, 1H), 7.33-7.30 (m, *J* = 19.0 Hz, 5H), 6.90 (d, *J* = 17.4 Hz, 1H), 6.23 (d, *J* = 36.6 Hz, 1H), 3.85 (s, 3H), 3.67 (m, 2H,), 2.93 (t, *J* = 19.2 Hz, 2H).

Compound **54**: Methyl 2-chlorocinnamate (155.4 mg, 0.76 mmol), phenylethylamine (225.5 mg, 2.4 mmol), and diisopropylethylamine 0.2 mmol were employed to give (*E*)-3-(2-chlorophenyl)-*N*-phenethylacrylamide (55%); light yellow solid; m.p. 108.2-115.3 °C; R_f (30% EtOAc/n-hexane) 0.50; FT-IR (neat) 3291, 1653, 1619, 1338, 577 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 7.94 (d, *J* = 20.4 Hz, 1H), 7.53-7.23 (m, 9H), 6.37 (d, *J*= 19.9 Hz, 1H), 3.72 (m, 2H), 2.94 (t, *J* = 17.4 Hz, 2H).

Compound **55**: methyl 2-(4-hydroxyphenyl)acetate (194.0 mg, 1.14 mmol), phenylethylamine (337.5 mg, 2.74 mmol), and diisopropylethylamine 0.2 mmol were employed to give 4-fluoro-*N*-phenethylbenzamide (92%); clear brown viscous liquid; m.p. 85.5-98.2 °C; R_f (30% EtOAc/n-hexane) 0.56; FT-IR (neat) 3427, 3331, 1642, 1549, 1465, 1240, 604 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 8.37(s, 1H), 7.11 (s, 1H), 6.41- 6.34 (m, 5H) 6.25 (d, *J* = 17.7 Hz, 2H), 6.18 (d, *J*= 14.2 Hz, 1H), 5.84 (d, *J*= 13.3 Hz, 1H), 2.50 (s, 2H), 1.85 (m, 2H), 1.67 (m, 2H)

Compound **56**: (*E*)-methyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (202.3 mg, 0.96 mmol), phenylethylamine (280.1 mg, 2.31 mmol), and diisopropylethylamine 0.2 mmol were employed to give (*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-phenethylacrylamide (59%); gray white solid; m.p. 180.1-194.3 °C; R_f (30% EtOAc/n-hexane) 0.33; FT-IR (neat) 3412, 3229, 1643, 1545, 1501, 1201, 606 cm^{-1}; ^{1}H-NMR : (400 MHz, DMSO-d_6) **δ**H 8.29 (s, 1H), 7.83 (s, 1H), 7.26 -7.08 (m, 8H), 6.70-6.69 (m, 2H), 3.68 (d, *J*= 11.8 Hz, 2H), 3.40 (s, 3H), 2.65 (t, *J*= 19.1 Hz, 2H).

Compound **57**: 3-Hydroxy-4-methoxycinnamic acid (201.4 mg, 1.03 mmol), phenylethylamine (310.8 mg, 2.47 mmol), and diisopropylethylamine 0.2 mmol were

employed to give (*E*)-3-(3-hydroxy-4-methoxyphenyl)-*N*-phenethylacrylamide (52%); light brown crystal; m.p. 145.7-164.5 °C; R_f (50% EtOAc/n-hexane) 0.27; FT-IR (neat) 3437, 3306, 1652, 1558, 1263, 600 cm^{-1}; ^{1}H-NMR : (400 MHz, DMSO-d_6) : **δ**H 9.17 (s, 1H), 8.09 (s, 1H), 7.32 -7.20 (m, 8H) , 6.93 (d, *J*= 12.8 Hz, 1H), 6.40 (d, *J*= 18.9 Hz, 1H), 3.79 (s, 3H), 3.36 (m, 2H), 2.76 (t, *J*= 19.9 Hz, 2H).

Compound **58**: Methyl 3-methoxy-4-hydroxybenzoate (308.8 mg, 1.65 mmol), phenylethylamine (284.0 mg, 3.96 mmol), and diisopropylethylamine 0.2 mmol were employed to give 4-hydroxy-3-methoxy-*N*-phenethylbenzamide (61%); orange solid; m.p. 136.5-143.8 °C; R_f (30% EtOAc/n-hexane) 0.13; FT-IR (neat) 3455, 3119, 1624, 1552, 1511, 1103 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) **δ**H 7.43 (s, 1H), 7.36-6.88 (m, 8H), 6.22 (s, 1H), 3.95 (s, 3H), 3.71 (d, *J* = 23.6 Hz, 2H), 2.91 (d, *J* = 22.2 Hz, 2H).

Compound **59**: methyl 4-hydroxybenzoate (307.0 mg, 1.97 mmol), phenylethylamine (572.9 mg, 4.73 mmol), and diisopropylethylamine 0.2 mmol were employed to give 4-hydroxy-*N*-phenethylbenzamide (70%); white solid; m.p. 145.3-151.5°C; R_f (30% EtOAc/n-hexane) 0.40; FT-IR (neat) 3308, 3027, 1652, 1617, 1544, 1333, 604 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 7.61 (s, 1H), 7.55 (d, *J* = 20.1 Hz, 2H), 7.49-7.02 (m, 8H), 6.25 (d, *J* = 19.1Hz, 1H), 3.70 (m, 2H), 2.92 (t, *J* = 19.7 Hz, 2H).

Compound **60**: methyl 4-fluorobenzoate (307.2 mg, 1.67 mmol), phenylethylamine (482.9 mg, 4.67 mmol), and diisopropylethylamine 0.2 mmol were employed to give (E)-3-(4-fluorophenyl)-*N*-phenethylacrylamide (45%); white solid; m.p. 145.3-151.5°C; R_f (30% EtOAc/n-hexane) 0.40; FT-IR (neat) 3308, 3027, 1652, 1617, 1544, 1333, 604 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 7.61 (s, 1H), 7.55 (d, *J* = 20.1 Hz, 2H), 7.49-7.02 (m, 8H), 6.25 (d, *J* = 19.1Hz, 1H), 3.70 (m, 2H), 2.92 (t, *J* = 19.7 Hz, 2H).

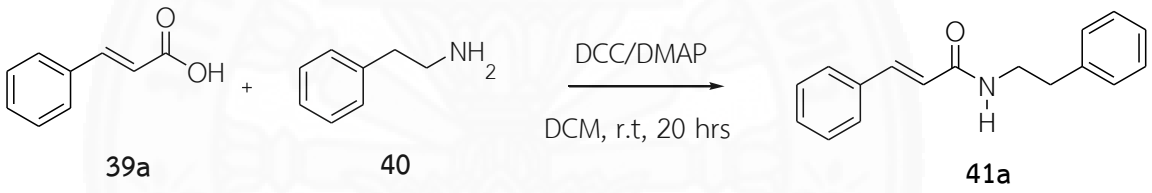
Compound **61**: (*E*)-methyl 3-(4-fluorophenyl)acrylate (301.4 mg, 1.95 mmol), phenylethylamine (573.9 mg, 4.67 mmol), and diisopropylethylamine 0.2 mmol were employed to give 4-fluoro-*N*-phenethylbenzamide (59%); white solid; m.p. 127.7-138.2 °C; R_f (30% EtOAc/n-hexane) 0.45; FT-IR (neat) 3350, 1642, 1605, 1548, 1453, 699 cm^{-1};

^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 7.66 (d, *J* = 20.2 Hz, 2H), 7.38-7.05 (m, 7H), 6.03 (s, 1H), 3.75 (m, 2H), 2.96 (t, *J* = 19.0 Hz, 2H).

Compound **62**: (*E*)-methyl 3-(4-fluorophenyl)acrylate (123.0 mg, 0.59 mmol), phenylethylamine (184.1 mg, 1.42 mmol), and diisopropylethylamine 0.2 mmol were employed to give (*E*)-3-(4-nitrophenyl)-*N*-phenethylacrylamide (29%); orange solid; m.p. 177.2-198.6 °C; R_f (20% EtOAc/n-hexane) 0.42; FT-IR (neat) 3443, 3001, 2927, 1630, 1559, 1495, 1350 cm^{-1}; ^{1}H-NMR : (400 MHz, $CDCl_3$): **δ**H 8.36 (d, *J* = 17.1 Hz, 2H), 8.26 (d, *J* = 15.2 Hz, 2H), 8.26 (s, 1H), 7.84 (d, *J* = 18.3 Hz, 1H), 7.34-7.10 (m, 5H), 6.38 (d, *J* = 17.2 Hz, 1H), 3.38 (m, 2H), 2.80 (t, *J* = 17.2 Hz, 2H)

3.3.3 Acid activation *via* coupling reagent

1) DMAP/DCC

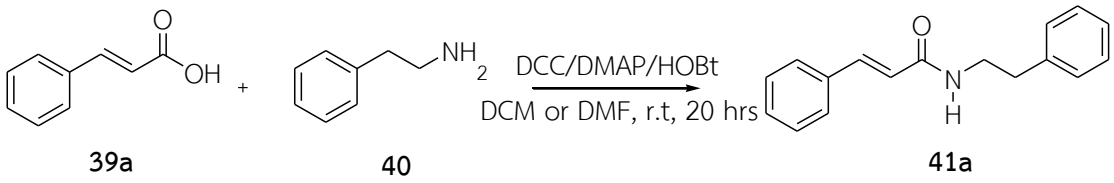


39a **40** **41a**

Scheme 3.3 Synthesis of amide by using DMAP/DCC as a coupling reagent.

General procedure: A solution of *N*, *N*′-dicyclohexylcarbodiimide (0.81 mmol, 1.2 equiv.) in methylene chloride (0.5 mL) was added dropwise to a stirred mixture of cinnamic acid (**39a**) (0.675 mmol, 1 equiv.), phenyl ethylamine (**40**) (0.81 mmol, 1.2 equiv), and 4-dimethylaminopyridine (0.0675 mmol, 0.1 equiv.) in methylene chloride (1.0 mL). The mixture was stirred at rt for 24 hours, then the white solid was filtered with a plug of cotton. The methylene chloride was added to the filtrate and the solution was washed with 0.5 M HCl and saturated sodium bicarbonate, respectively. The organic layer was dried using sodium sulfate and concentrated to dryness. The residue was purified by flash column chromatography eluting with 0 to 40% ethyl acetate in hexane to obtain the desired amide (**41a**).

2) Using DMAP/DCC and HOBt as a coupling reagent

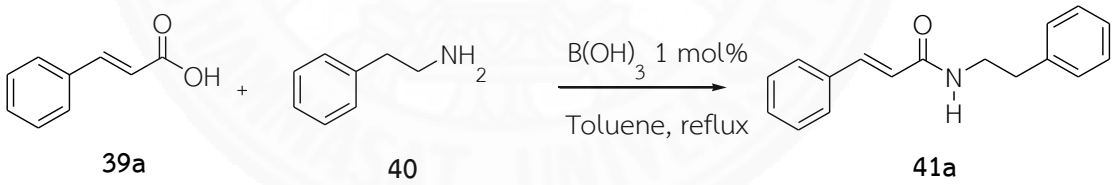


39a **40** **41a**

Scheme 3.4 Synthesis of amide by using DMAP/DCC and HOBt as a coupling reagent.

General procedure: A solution of *N*, *N*′-dicyclohexylcarbodiimide (0.138 g, 0.675 mmol) in methylene chloride (1.0 mL) was added dropwise to a stirred mixture of cinnamic acid (**39a**) (0.100 g, 0.675 mmol), phenyl ethylamine (**40**) (0.123 g, 1.0125 mmol), and 4-dimethylaminopyridine (0.0825 g, 0.675 mmol) in methylene chloride (2.0 mL). The mixture was stirred at rt for 24 hours, then the white solid was filtered with a plug of cotton. The methylene chloride was added to the filtrate and the solution was washed with 0.5 M HCl and saturated sodium bicarbonate, respectively. The organic layer was dried using sodium sulfate and concentrated to dryness. The residue was purified by flash column chromatography eluting with 0 to 40% ethyl acetate in hexane to obtain the desired amide (**41a**).

3) Amide synthesis *via* boric acid



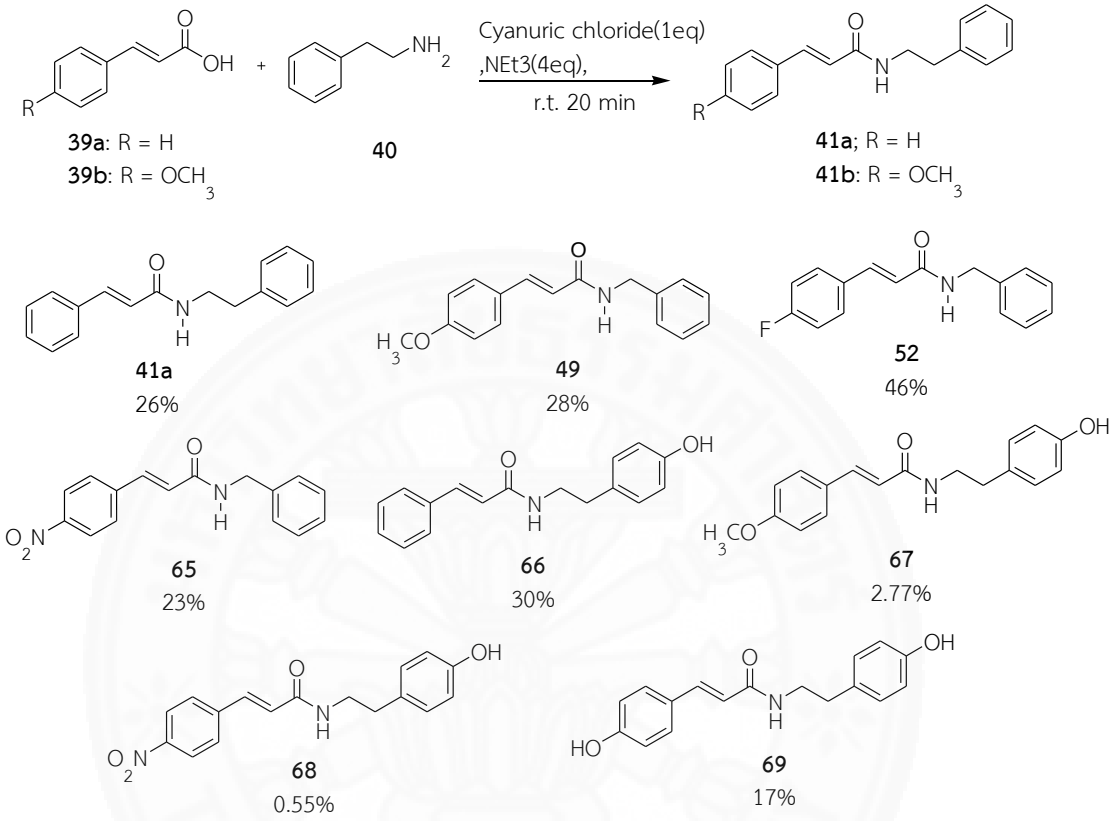
39a **40** **41a**

Scheme 3.5 Synthesis of amide **41a** by using boric acid as a catalyst.

General procedure: The reaction vessel is charged with cinnamic acid (**39a**) (0.0741 g, 0.5 mmol), boric acid (0.0003 g, 0.005 mmol), and 1.5 mL of toluene. To the stirred colorless reaction mixture is added phenyl ethylamine (**40**) (0.0667 g, 0.55 mmol) in one portion. The reaction mixture is heated to reflux for 3 hours. The mixture was cooled down to at room temperature and then is poured while stirring into 50 mL of hexanes leading to the immediate precipitation of a white solid. Stirring is continued for an additional 10 min and then the precipitate is filtered off with suction. The

collected solid is washed with hexanes and then is dried at room temperature for 12 hours to produce compound (**41a**).

4) Amide synthesis *via* triazine-based coupling reagents.



Scheme 3.6 Synthesis of amide with cyanuric chloride as a coupling reagent.

General procedure: In a small round bottom flask charged with cinnamic acid (**39**) (0.161 g, 1.08 mmol), phenyl ethylamine (0.076 g, 0.92 mmol), cyanuric chloride (0.100 g, 0.54 mmol), triethylamine (0.109 g, 1.08 mmol), and dichloromethane (3mL) were mechanically stirred overnight at room temperature. After completion of the reaction monitored by TLC, the reaction mixture was washed with 1M hydrochloric acid (2 x 10 mL), 1M sodium hydroxide (2 x 20 mL), and water (2 x 20 mL). Then, the solvent was dried and removed under reduced pressure and purified by flash column chromatography to produce compound **41**; ; white crystal; m.p. °C; R_f (30% EtOAc/n-hexane) 0.56; FT-IR (neat) 3526, 3495, 3092, 3090, 3017, 2968, 1610, 1257 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.04 (s, 1H), 7.03 (d, *J* = 6.01 Hz, 1H), 6.86-6.83 (m, 8H), 6.64 (d, *J* = 12.81 Hz, 1H), 3.78 (s, 3H).

Compound **49b:** : 4-Methoxycinnamic acid (192.4 mg, 1.08 mmol) and benzylamine (127.3 mg, 1.18 mmol), cyanuric chloride (99.6 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give *N*-benzyl-3-(4-fluorophenyl)prop-2-enamide; light yellow solid; m.p. 118-128 °C; R_f (30% EtOAc/n-hexane) 0.37; FT-IR (neat) 3291, 3033, 3000, 3033, 3000, 1606, 1257,1051 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.38-7.19 (m, 4H), 6.83-6.80 (m, 5H), 6.25-6.19 (d, *J* = 22.85 Hz, 1H), 4.51-4.49 (d, *J* = 13.86 Hz, 1H), 3.89 (s, 3H), 3.76-3.73 (d, *J* = 10.36 Hz, 2H)

Compound **52:** 4-Fluorocinnamic acid (179.4 mg, 1.08 mmol), benzylamine (115.7 mg, 1.08 mmol), cyanuric chloride (99.6 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give were employed to give N-benzyl-3-(4-fluorophenyl)prop-2-enamide; light yellow solid; m.p. 118-128 °C; R_f (30% EtOAc/n-hexane) 0.37; FT-IR (neat) 3283, 3091, 3060, 2930, 2865, 1659, 1230 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.70-7.60 (br m, 2H), 7.50-7.45 (br m, 1H), 7.40 (s, 2H), 7.37 (s, 1H), 7.33 (s, 2H), 6.35 (br s, 1H), 5.85 (br s ,1H), 4.60 (s, 1H)

Compound **65:** 4-Nitrocinnamic acid (208.6 mg, 1.08 mmol), benzylamine (127.3 mg, 1.18 mmol), cyanuric chloride (99.6 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give *N*-benzyl-3-(4-nitrophenyl)prop-2-enamide; light yellow solid; m.p. 118-128 °C; R_f (30% EtOAc/n-hexane) 0.30; FT-IR (neat) 3301, 3091, 2976, 2932. 1612, 1590, 1345 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 8.76 (s, 1H), 8.27-8.26 (m, 4H), 7.85-7.83 (d, *J*=12.21 Hz, 1H), 7.30-7.25 (m, 5H), 6.80-6.90 (d, *J*=18.80 Hz, 1H), 4.43 (d, *J* =14.28 Hz, 1H)

Compound **66:** cinnamic acid (160mg, 1.08 mmol), tyramine (148.2 mg, 1.08 mmol), cyanuric chloride (100 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give *N*-cinnamoyltyramine; light yellow solid; m.p. 188-189 °C; R_f (20% EtOAc/n-hexane) 0.70; ^{1}H-NMR (300 MHz, DMSO-d_6) **δ**H 9.18 (s, 1H), 8.15 (t, *J* = 5.7 Hz, 1H), 7.56 (d, *J* = 1.5 Hz, 1H), 7.54 (s, 1H), 7.43-7.34 (m,

4H), 7.03 (s, 1H), 7.00 (s, 1H), 6.69 – 6.59 (m, 3H), 3.34 (t, *J* = 10.05 Hz, 2H), 2.65 (t, *J* = 7.35 Hz, 2H).

Compound **67:** 4-Methoxycinnamic acid (192.4 mg, 1.08 mmol), tyramine (100 mg, 1.18 mmol) , cyanuric chloride (99.6 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give 2-propenamide, *N*-[2-(4-hydroxyphenyl)ethyl]-3-(4-methoxyphenyl)-(0%); brown liquid.

Compound **68:** 4-Nitrocinnamic acid (206.9 mg, 1.08 mmol), tyramine (108.5 mg, 1.18 mmol) , cyanuric chloride (99.6 mg, 0.54 mmol), triethylamine (218.6 mg 2.16 mmol), and dichloromethane (1 mL) were employed to give (2*E*)-*N*-[2-(4-Hydroxyphenyl)ethyl]-3-(4-nitrophenyl)-2-propenamide; light yellow solid.

Compound **69:** coumaric acid (100 mg, 0.61 mmol), tyramine (125.5 mg, 0.915 mmol) , cyanuric chloride (74.5 mg, 0.61 mmol), triethylamine (125.9 mg 0.61 mmol), and dichloromethane (1 mL) were employed to give 4-coumaroyl tyramine; white solid; m.p. 234-236 °C; R_f (50% EtOAc/n-hexane) 0.72; ^{1}H-NMR (400 MHz, DMSO-d_6) **δ**H 7.34 (d, *J* = 15.6 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.68 (2H, d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 6.28 (d, *J* = 15.6 Hz, 1H), 3.36 (t, *J* = 7.5 Hz, 2H), 2.65 (t, *J* = 7.5 Hz, 2H).

3.3.4 Microwave-assisted synthesis of amide

1) Using boric acid as a catalyst

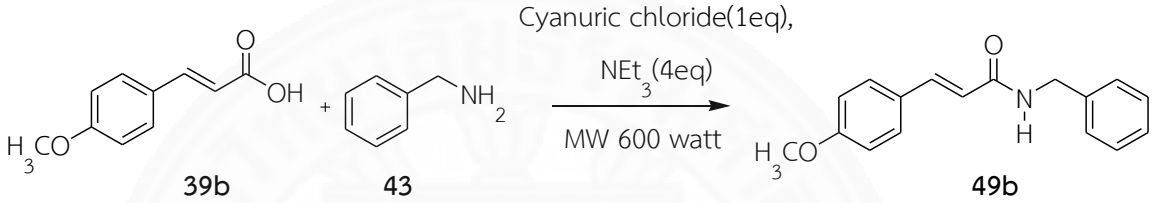
39b **43** **49b**

Scheme 3.7 Amide synthesis using boric acid as a coupling reagent by MWI.

General procedure: The reaction vessel is charged with cinnamic acid (**39b**) (0.0741 g, 0.5 mmol), boric acid (0.0003 g, 0.005 mmol), and 1.5 mL of toluene. To the stirred colorless reaction mixture one portion of benzylamine (0.0667 g, 0.55 mmol) is added. The reaction mixture was irradiated for 1 min. in a microwave oven at power

600 watt. The mixture was cool down at room temperature and then is poured while stirring into 20 mL of hexanes leading to the immediate precipitation of a white solid. Stirring is continued for an additional 10 min. and then the precipitate is filtered off with suction. The collected solid is washed with of hexanes and then is dried at room temperature for 12 hours to produce compound **49b**. R_f: 0.38 (30%EA/Hex), ^{1}H-NMR (400MHz, $CDCl_3$): 7.19-7.38 (m, 4H), 6.80-6.83 (m, 5H), 6.19-6.25 (d, *J* = 22.85 Hz, 1H), 4.49-4.51 (d, *J* =13.86 Hz, 1H), 3.89 (s, 3H), 3.74-3.76 (d, *J* = 10.36 Hz, 2H)

2) Amide synthesis with cyanuric chloride

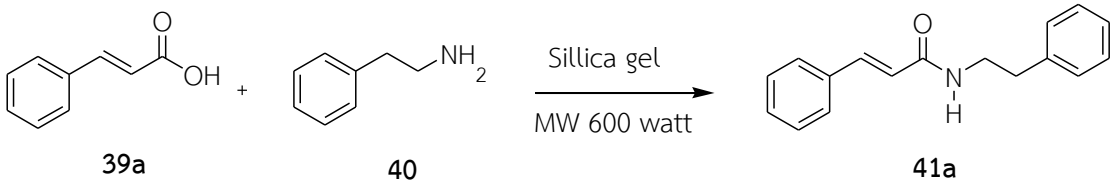


Scheme 3.8 Synthesis of amide **49b** by cyanuric chloride under microwave irradiation.

General procedure: In a small round bottom flask charged with cinnamic acid (**39b**) (0.161 g, 1.08 mmol), phenyl ethylamine (0.076 g, 0.92 mmol), cyanuric chloride (0.100 g, 0.54 mmol), triethylamine (0.109 g, 1.08 mmol), and dichloromethane (3mL) was irradiated for 6 min (or every 2 min. intervals until the reaction is complete by TLC) in a microwave oven at power 600 watt. After completion of the reaction, the reaction mixture was washed with 1M hydrochloric acid (2 x 10 mL), 1M sodium hydroxide (2 x 20 mL), and water (2 x 20 mL), respectively. Then, the solvent was dried using sodium sulfate and the dried solvent was removed under reduced pressure. The residue was purified by flash column chromatography to produce compound **49b**.

3.3.5 Microwave-assisted synthesis with silica gel.

1) Non-using a catalyst



Scheme 3.9 Synthesis of amide **41a** by Microwave-assisted synthesis with silica gel.

General procedure: In a small round bottom flask contained with cinnamic acid (**39a**) (0.222 g, 1.5 mmol), phenyl ethylamine (0.226 g, 1.8 mmol) was dissolved with ethyl acetate (15mL) and silica gel (1 g) were added, then all were mixed together. The mixture was evaporated to get the white solid. The solid was irradiated for 60 min in a microwave oven at power 600 watt (heated for 4 min. alter the rest for 4 minutes. then replete until 60 min. of microwave irradiation has been completed). After completion of the reaction, the solid was washed with ethyl acetate (2 x 30 mL) and sonicated for 20 min. Filtration to remove of silica gel and filtrate was washed with 1M hydrochloric acid (2 x 10 mL) and 1M sodium hydroxide (2 x 20 mL),respectively. Then, the solvent was dried using sodium sulfate and the dried solvent was removed under reduced pressure. The residue was purified by flash column chromatography to produce compound **41a**.

2) Using DMAP/DCC and HOBt as a coupling reagent

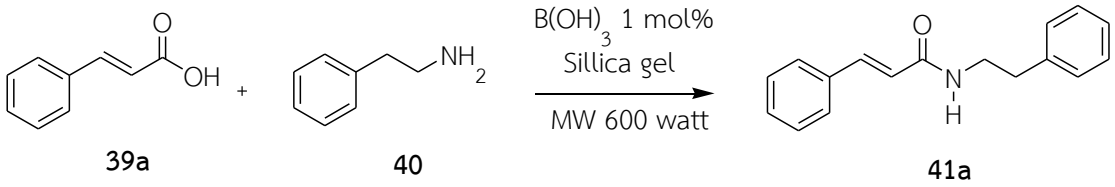
39a **40** **41a**

Scheme 3.10 Microwave-assisted synthesis with silica gel by using DMAP/DCC and HOBt as a coupling reagent.

General procedure: In a small round bottom flask charged with cinnamic acid (**39a**) (0.130 g, 0.88 mmol), phenyl ethylamine (0.128 g, 1.056 mmol), *N*, *N*´-dicyclohexylcarbodiimide (0.181 g, 0.88 mmol), 4-dimethylaminopyridine (0.108 g, 0.88 mmol), and 1-hydroxybenzotriazole (HOBt) (0.147 g, 0.96 mmol) which was dissolved with dichloromethane (15mL) and silica gel (1 g) were added, then all were mixed together. The mixture was evaporated to get the white solid. The solid was irradiated for 60 min. in a microwave oven at power 600 watt (heated for 4 min. alter the rest for 4 min. then replete until 60 min. of microwave irradiation is completed). After completion of the reaction, the solid was soaked with dichloromethane (2 x 30 ml) and sonicated for 20 min. The organic extract was filtered to remove silica gel and the filtrate was washed with 1M hydrochloric acid (2 x 10 mL) and 1M sodium hydroxide

(2 x 20 mL). The dichloromethane extract was dried using sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography to obtain compound **41a**.

3) Using boric acid as a catalyst

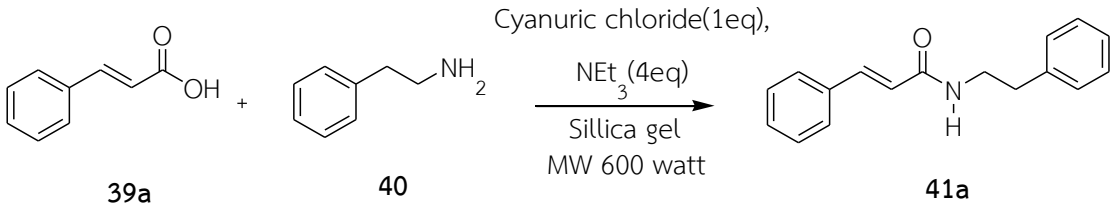


39a **40** **41a**

Scheme 3.11 Microwave-assisted synthesis with silica gel by using boric acid as a catalyst.

General procedure: In a small round bottom flask, cinnamic acid (**39a**) (0.222 g, 1.5 mmol), phenyl ethylamine (0.217 g, 1.8 mmol), and boric acid (0.0009 g, 0.015 mmol) was dissolved with methanol (15mL) and silica gel (1 g) were added, then all were mixed together. The mixture was evaporated to get the white solid. The solid was irradiated for 60 min. in a microwave oven at power 600 watt (heated for 4 min. alter the rest for 4 min. then replete until 60 min. of microwave irradiation). After completion of the reaction, the solid was washed with dichloromethane (2 x 30 ml) and sonicated for 20 min. Filtration was used to remove of silica gel and the filtrate was washed with 1M hydrochloric acid (2 x 10 mL) and 1M sodium hydroxide (2 x 20 mL), respectively. Then, the solvent was dried using sodium sulfate and the dried solvent was removed under reduced pressure. The residue was purified by flash column chromatography to produce compound **41a**.

4) Using cyanuric chloride as a catalyst



39a **40** **41a**

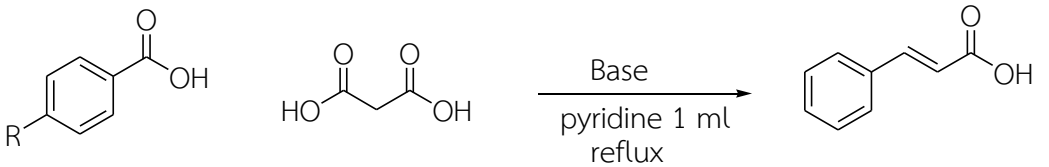
Scheme 3.12 Microwave-assisted synthesis with silica gel by using cyanuric chloride as a catalyst.

General procedure: In a small round bottom flask charged with cinnamic acid (**39a**) (0.161 g, 1.08 mmol), phenyl ethylamine (0.185 g, 1.35 mmol), cyanuric chloride (0.100 g, 0.54 mmol), and triethylamine (0.219 g, 2.16 mmol) was dissolved with dichloromethane (15mL) and silica gel (1 g) were added, then all were mixed together. The mixture was evaporated to get the white solid. The solid was irradiated for 60 min. in a microwave oven at power 600 watt (heated for 4 min. alter the rest for 4 min. then replete until 60 min. of microwave irradiation is completed). After completion of the reaction, the solid was soaked with dichloromethane (2 x 30 ml) and sonicated for 20 min. Filtration was done to remove of silica gel and the filtrate was washed with 1M hydrochloric acid (2 x 10 mL) and 1M sodium hydroxide (2 x 20 mL), respectively. Then, the solvent was dried using sodium sulfate and the dried solvent was removed under reduced pressure. The residue was purified by flash column chromatography to produce compound **41a**.

During the amide synthesis, some acids were not available in our lab so the synthesis of cinnamic acid derivatives was studied. We begin with the synthesis of cinnamic acid using a well-known procedure, Knoevenagel–Doebner condensation described in the below section. After we were familiar with the procedure we then attempted this reaction under microwave irradiation with catalysts as described in the literature.[31,32] Later, we hypothesized that the synthesis of phenylpropanoid amide derivatives could be constructed directly by two-step, one-pot reaction from aromatic aldehydes and amine under microwave irradiation. To prove the hypothesis, the two-step, one-pot conditions were performed.

3.3.6 Cinnamic acid synthesis

1) Knoevenagel–Doebner condensation[36]



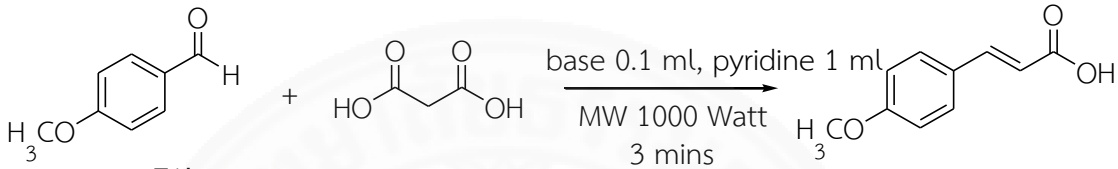
71a; R = H
71b; R = OCH_3

39a; R = H
39b; R = OCH_3

Scheme 3.13 Knoevenagel–Doebner condensation of cinnamic acid (**39**) by reflux.

General procedure: Aldehyde (**71**) (0.125 g, 1.2 mmol), malonic acid (0.106 g, 1 mmol), and piperidine (0.1 mL, 1 mmol) were dissolved by pyridine (1mL) in a round bottom flask. The reaction mixture using a refluxing system for 24-72 hours. Then, the reaction was poured in cold 1M hydrochloric acid to get the white precipitates. The solid was filtered and purified by flash column chromatography using hexane and ethyl acetate as the eluent. The structure was characterized by ^{1}H-NMR spectrometry.

2) Knoevenagel–Doebner condensation under microwave irradiation[31]

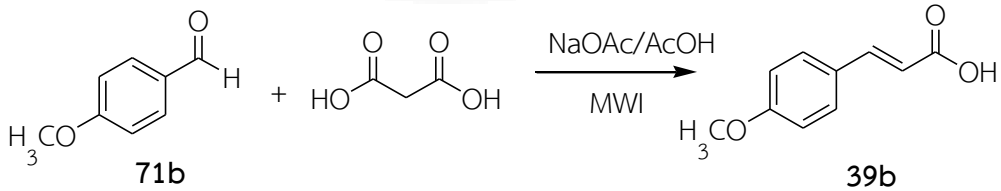


71b **39b**

Scheme 3.14 Knoevenagel–Doebner condensation of cinnamic acid (**39b**) by Microwave irradiation.

General procedure: A mixture of *p*-anisaldehyde (**71b**) (0.136 g, 1 mmol), malonic acid (0.416 g, 4 mmol), pyridine (1.0 mL) and piperidine (0.1 mL) in a vial was irradiated for 3-26 minute intervals in a microwave oven at power 600 watt. The mixture was cooled. The excess pyridine was neutralized by the addition of cold 1:1 hydrochloric acid. The precipitated acid was filtered and washed with cold water. R_f: (50%EtOAc/Hex) 0.57; ^{1}H-NMR (400MHz, $CDCl_3$) **δ**H 7.70-7.80 (d, 2H) 7.55 (d, 1H), 6.95 (d, 2H), 6.35 (d, 1H), 3.95 (s, 1H).

3) Microwave irradiation using sodium acetate[37]



71b **39b**

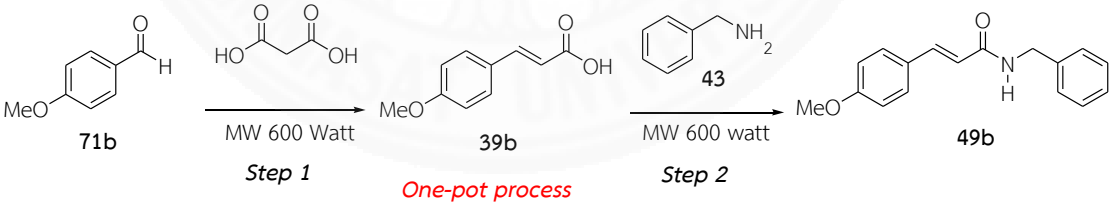
Scheme 3.15 Sodium acetate catalyzed of cinnamic acid **(39b)** by Microwave irradiation.

General procedure: In a small round bottom flask, malonic acid (0.383 g, 3.68 mmol), sodium acetate (0.076 g, 0.92 mmol), and acetic acid (0.223 g, 3.68 mmol) were

mechanically stirred to a homogeneous mixing (sticky solid). To this solution *p*-anisaldehyde (**71b**) (0.136 g, 1 mmol) was added and solution mixture was irradiated with microwave irradiation at power 600 watt at 40 °C for the appropriate time (TLC check). At this stage, the reaction mixture changes to pale yellow colored viscous liquid. Obtained viscous liquid quickly solidified at room temperature to offer solid.

3.3.7 Two-step, one-pot synthetic method

We investigated to find a simple and efficient, two-step, one-pot synthetic method for the direct conversion of aromatic aldehydes to cinnamate amides using microwave irradiation. We have divided the amide synthesis into two processes. The first step was the synthesis of cinnamic acid derivatives. Here we began with a Doebner modification condition in the presence of a base such as NH_4OH, NH_4COOCH_3 and piperidine. The condition proceeded rapidly under microwave irradiation using water, triethylamine, or pyridine as solvent. The attempts to synthesize the cinnamic acid derivatives catalyzed by sodium acetate were also performed. The second step used an excess of amine in the presence of coupling reagent including boric acid and cyanuric chloride. The investigation of this two-step synthesis was then studied with and without purification of the cinnamic acid derivatives synthesized from the first step.



Scheme 3.16 Synthesis of amide from aldehyde; two-step, one-pot synthetic method.

1) Doebner modification

General procedure: In the first step, a mixture of *p*-anisaldehyde (**71b**) (0.136 g, 1 mmol), malonic acid (0.416 g, 4 mmol), pyridine (1.0 mL) and piperidine (0.1 mL) was irradiated for 3 mins in a microwave oven at power 600 watt. Then, the cinnamic acid

from the first step was combined with amine to synthesize amide bond in the second step.

2) Catalyzed by sodium acetate

General procedure: In the first step, a small round bottom flask mechanically stir malonic acid (0.161 g, 1.08 mmol), sodium acetate and acetic acid (0.073 mol) (1:1) were mechanically stirred to a homogeneous mixing (sticky solid). To this solution the substituted aromatic aldehyde (0.073 mol) was added and the solution mixture was irradiated with the microwave synthesizer at power 600 watt at 40°C for the appropriate time (TLC check). Then, the cinnamic acid from the first step was combined with amine to synthesize amide bond in the second step.

3.4 Biological activity

Coumaroyl tyramine derivatives exhibit several biological activities such as anti-inflammatory,[11] antioxidant activity,[11] acetylcholinesterase enzyme inhibition,[11] anti-adipogenic,[10] and pancreatic lipase[10] activities. Therefore, we wanted like to evaluate the biological activity of the synthesized compounds such as phytotoxicity, insecticidal activity, antifeedant activity, antioxidant activity and antipancreatic lipase activity under the collaboration with Asst. Prof. Dr. Sornkanok Vimolmangkang.

3.4.1 Phytotoxicity of lettuce seeds[38]

3.4.1.1 Preparation of sample 0.5 mg/mL

The sample solution of coumaroyl tyramine derivatives was prepared by accurately weighed coumaroyl tyramine derivatives 5.0 mg and was dissolved in 10.0 mL of acetone. 0.25 mg/mL of sample solution were obtained by appropriate dilution of the stock solution with the same solvent and using an acetone as a control.

3.4.1.2 Assay method

Phytotoxicity of lettuce seeds was determined by measuring length of leaves, haulm, and roots. First, filter paper (110nm) was placed in 15x100 mm of cultured dish then pipette 0.25 and 0.5 mg/mL of sample solution 2 mL into the dish (repeat 2 times) and wait until volatile. Second step, pipette 2 mL of distilled water after that 10 seeds of lettuce seeds was added and cultured for 6 days in culture cabinet. The

final step was measuring the length of leaves, haulm, and roots and then finding the average. An acetone as a control (Figure 3.2).

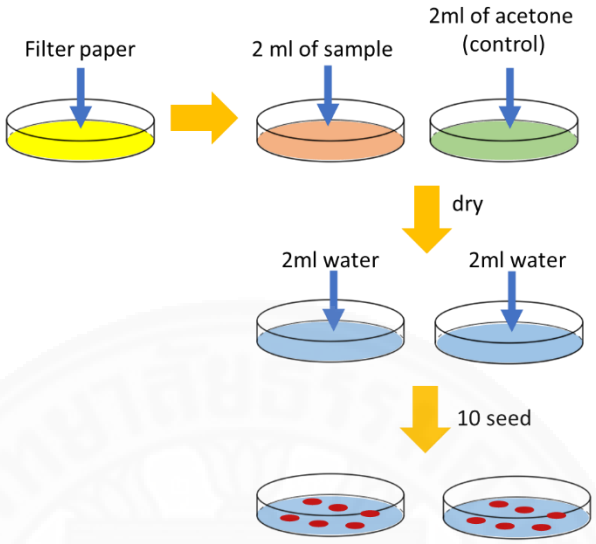


Figure 3.2 Phytotoxicity method of lettuce seeds.

3.4.2 Insecticidal assay of larvae of common cutworm.[39]

3.4.2.1 Preparation of sample 100 µg/mL

The sample solution of coumaroyl tyramine derivatives was prepared by accurately weighed coumaroyl tyramine derivatives 0.5 mg which were dissolved in 5.0 mL of acetone and using an acetone as a control.

3.4.2.2 Assay method

Insecticidal assay of the larvae of common cutworm, *Spodopteralitura* was determined by counting the number of live worms. First, prepare the samples into a vial and evaporate with nitrogen gas so that the coating is on bottom of vial and about 1 cm from the bottom of vial. Second, bring 5 vegetable worms, phase 1 to the vial and leave for 6 hours. The final step was to count the number of live worms and repeat for 4 vials/samples. An acetone as a control was used as shown in Figure 3.3.

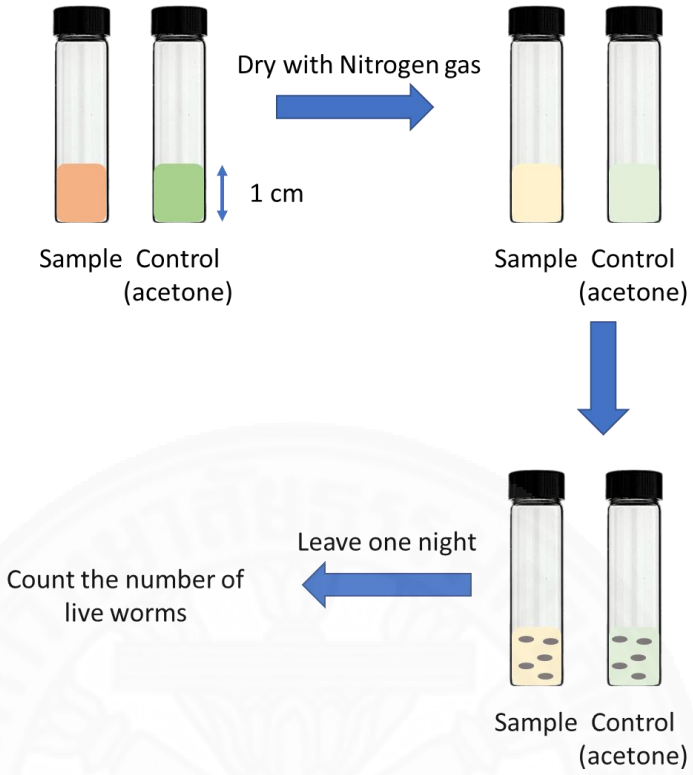


Figure 3.3 Insecticidal assay of common cutworm.

3.4.3 Antifeedant activity[40]

3.4.3.1 Preparation of sample 0.5 mg/dish

The sample solution of coumaroyl tyramine derivatives was prepared by accurately weighed coumaroyl tyramine derivatives 3.0 mg which was dissolved in 0.6 mL of acetone, using an acetone as a control.

3.4.3.2 Antifeedant activity process

Antifeedant process of the vegetable worms, phase 3 was determined by calculating the antifeedant index. First, the sweet potato leaves were cut into a round, 2 cm diameter size, soaked in water and then filter paper (110 nm) was placed in a 25x150 mm cultured dish and distill water was added to the dish (repeat twice). In the second step, the cut sweet potato leaves were put into 4 sides of culture dish: top, bottom, left, and right, then 100 µL of sample pipette was placed on the top and bottom potato leaves. After that 100 µL of acetone pipette was placed on the left and right parts as a control as shown in Figure 3.4. In the next step, 10 vegetable worms, phase 3 were added into the culture dish and left for 2 days.

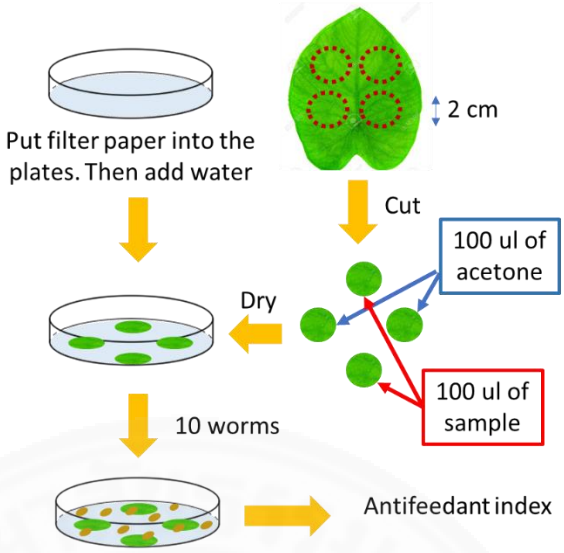


Figure 3.4. Antifeedant activity process.

The final step was measuring areas of sweet potatoes that were not eaten by the worms to calculate the antifeedant index using the following equation:

$$\% \text{ insect antifeedant activity} = 100 - 2((T-C)/T)$$

where T is the area of the leaf that contains the sample and C is the area of the leaf that contains the control.

3.4.4 Determination of free radical scavenging activity[41]

The DPPH (2 ,2 - diphenyl-1 - picrylhydrazyl) assay has been widely used to determine the free radical scavenging activity. In this work, we studied an antioxidant activity of coumaroyl tyramine derivatives by free radical scavenging activity. Gallic acid was used as a positive control.

3.4.4.1 Preparation of reagents and sample

(1) DPPH (50 μM)

DPPH solution was prepared by accurately weighed DPPH 2.0 mg which was then dissolved in a 100 mL volumetric flask with methanol.

(2) Gallic acid (1000 μg/mL)

Gallic acid solution was prepared by accurately weighed Gallic acid 1.0 mg which was then dissolved in a 10 mL volumetric flask with methanol.

(3) Sample (coumaroyl tyramine derivatives, 1000 μg/mL)

The stock solution of coumaroyl tyramine derivatives was prepared by accurately weighed coumaroyl tyramine derivatives 1.0 mg which was then dissolved in 1.0 mL of methanol. Working solutions (at concentrations of 1000, 500, 250, 125, 62.5, 31.25 μg/mL) were obtained by appropriate dilution of the stock solution with the same solvent.

3.4.4.2 Assay method

Free radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, resulting in 20 μL of the test sample solutions (6 concentrations), and 180 μL of DPPH (50 μM). After the mixture was shaken slowly and incubated for 30 min at room temperature, and the absorbance at 510 nm using a microplate reader was then measured.

The scavenging activity was calculated by determining the decrease in the absorbance at a different concentration by using the following equation:

$$\% \text{ inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) x100$$

where $A_{control}$ is the absorbance of blank sample (no added sample), and A_{sample} is the absorbance in the presence of sample.

3.4.4 Determination of antipancreatic lipase activity

3.4.5.1 Preparation of reagents and sample

(1) Tris buffer 0.1M pH 8.2 for 40 mL

Tris buffer prepared by accurately weighed 631.2 mg, dissolved in 20.0 mL of water, adjusted with 1N HCl to pH 8.0-8.2.

(2) CH_3COONa 50 mM pH 5.0 for 5.0 mL

CH_3COONa prepared by accurately weighed 20.508 mg, dissolved in 3-4 mL water, adjusted pH with 1 N HCl until pH 5.0.

(3) 10mM p-Nitrophenyl laurate for 1 mL [MW=321.417 g/mol]

p-Nitrophenyl laurate prepared by accurately weighed 3.21 mg, dissolved in CH_3COONa 0.9 mL and Triton X-100 0.1 mL and warm on dry bath at 60 ℃ until *p*-nitrophenyl laurate is dissolved.

(4) Lipase 0.1 mg/mL for 10mL

Lipase solution prepared by accurately weighed 0.2 mg, dissolved in 20 mL tris buffer and centrifuge with 12,000 rpm at 4 °C for 5 minutes.

(5) Stock sample 2 mg/mL

10 mM of stock sample prepared by accurately weighed 10 mg, dissolved in a 1 mL 100% DMSO. stock sample 2 mM were obtained by appropriate dilution of the stock solution with 20% DMSO.

(6) Orlistat 0.26 mg/1 mL for 1 mL

Orlistat prepared by accurately weighed 0.26 mg, dissolved in a 1 mL 20% DMSO.

3.4.5.2 Assay method

Lipase inhibition activity was determined by spectrophotometry. 5 μL of the test samples solution, and 90 μL of Lipase solution (0.1 mg/mL). After the mixture was shaken slowly and incubated for 30 min at 37 °C. 5 μL of 10 mM *p*-NPL was added to the enzyme mixture and incubated for 30 min at 37 °C, and the absorbance at 405 nm using a microplate reader was then measured.

The lipase inhibition activity was calculated by determining the decrease in the absorbance at a different concentration by using the following equation:

$$\% \text{ inhibition} = \left[1 - \frac{(B-b)}{(A-a)}\right] x100$$

where A is the activity without inhibitor, a is the negative control without inhibitor, B is the activity with inhibitor, and b is the negative control with inhibitor.

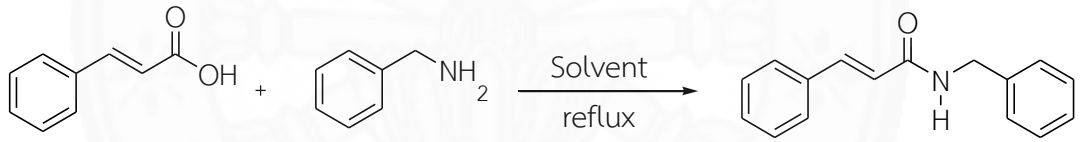
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Introduction

We first investigated to find a simple and efficient synthetic method for the synthesis of phenylpropanoid amide or *p*-coumaroyl tyramine derivatives (Figure 3.1). After getting the optimum condition, the derivatives were then synthesized. The evaluation of the biological activities in this study included phytotoxicity, insecticidal activity, antifeedant activity, antioxidant activity, and antipancreatic lipase activity.

4.2 Amide Synthesis

4.2.1 Thermal direct condensation



39a **43** **49a**

Scheme 4.1. Amide bond formation by direct thermal reaction.

We first investigated the synthesis of amide using simple thermal direct condensation. The reaction was heated to reflux using dean stark apparatus to remove the condensed water. Xylene was used as solvent to provide the high temperature. The results shown in Table 4.1 indicated the excess of amine is necessary to gain satisfactory yield. The optimum condition from entry **3** was applied to generate the amide derivatives **49-53** shown in Figure 4.1 This condition provides high yield of the amide derivatives except for the compound **53**.

Table 4.1 The optimization for amide bond formation by direct thermal reaction.

Entry	Carboxylic acid (eq)	Amine (eq)	Solvent	Temperature (°C)	Time(h)	%Yield
1	1	1	Xylene	140	4	39
2	1	1.5	Xylene	140	4	41
3	1	4	Xylene	140	18	50-84
4	1	1	Toluene	110	5	42-72

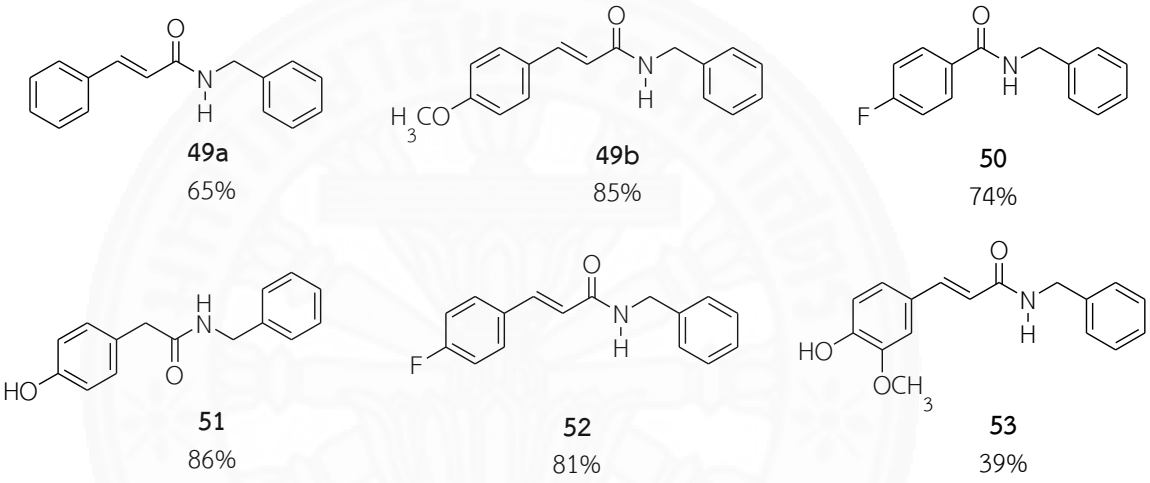
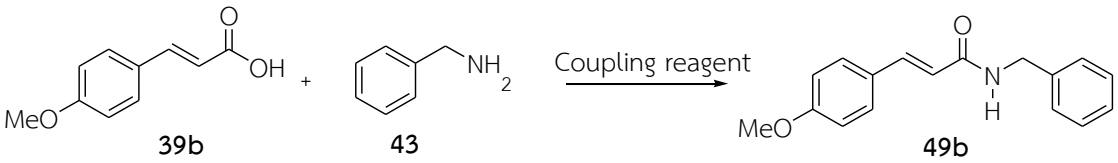


Figure 4.1 Structure of the *trans-N*-coumaroyltyramine derivatives from thermal direct condensation.

The results showed that the functional group at C3 position may be the reason for the higher percentage yield for the synthesis of compounds **49b**, **50**-**52** when comparing with compound **49a**, which not had the functional group at C3 position. The electron donating hydroxy and methoxy groups on the benzene ring of compounds **49b** and **51** may be the reason for the higher yield.

According to the results obtained, the amides **53** with methoxy and hydroxyl group at position 3 and 4 combined with benzylamine is lower yield compared with compounds **49b** and **51.**

4.2.2 Amide coupling method



Scheme 4.2 Amide bond formation by amide coupling method.

In this part, we optimize the conditions using amide coupling reagents: 1mol%$B(OH)_3$, DMAP/DCC, and cyanuric chloride. The yields were provided in Table 4.2. We found that the conditions using 1mol% boric acid (entry **1**) and cyanuric chloride (1eq), NEt_3 (4eq) (entry **2**) as a coupling reagent generated the cinnamate amide moderate to high yields. The common condition DMAP/DCC (entry **3**) was not practical for our synthesis. Therefore, we added HOBt in the reaction which using DMAP/DCC (entry **4**) resulted in a satisfactory yield of 91%.

Table 4.2 The optimization for amide bond formation by amide coupling method.

Entry	Carboxylic acid (eq)	Amine (eq)	Coupling reagent	Solvent	T emp. (°C)	Time(h)	%Yield
1	1	1.4	1mol%$B(OH)_3$	toluene	110	24	49-53
2	2	2	Cyanuric chloride (1eq), NEt_3 (4eq)	DCM	r.t.	3Days/6h	76-93
3	1	1.2	DMAP/DCC	DCM	r.t.	20	26
4	1	1.5	DMAP/DCC/HOBT	DMF	r.t.	2	91
5	1	1.5	DCC/HOBT	DMF	r.t.	9days	31
6	1	1.5	DMAP/HOBT	DMF	r.t.	24	-
7	1	1.5	DMAP	DMF	r.t.	24	-
8	1	1.5	DCC	DMF	r.t.	24	24
9	1	1.5	HOBT	DMF	r.t.	24	3

The desired cinnamate amides can be prepared employing cyanuric chloride (1 equiv.) as a coupling reagent in the presence of NEt_3 (4 equiv.) at room temperature

using dichloromethane as the solvent (entry **2**). This method gave the amide derivatives **41a, 49b, 52, and 65-69** shown in Figure 4.2. This condition provided moderate yield of the amide derivatives except for the compounds **67** and **68**.

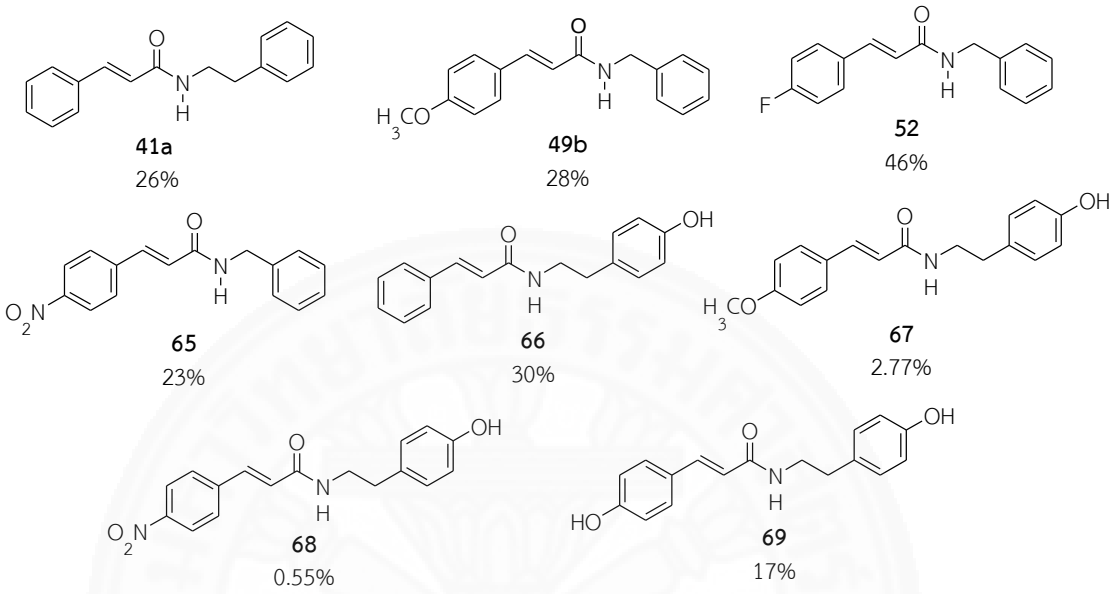
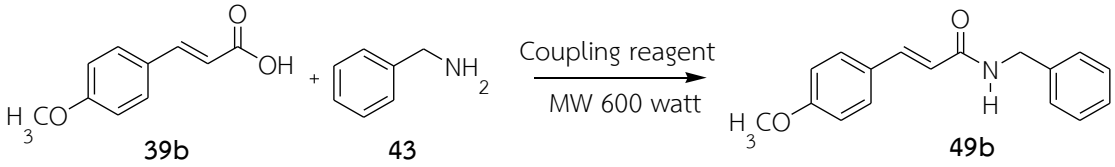


Figure 4.2 Structure of the coumaroyl tyramine derivatives from coupling reagent method.

From the synthesis of the amide derivatives via activating agent cyanuric chloride, it was found that amide products can be synthesized, but in a lower yield than the amide produced from direct thermal condensation. This may be because of the solubility issue as the reactants cannot be completely soluble in dichloromethane. We are in the progress of finding a better solvent for this reaction. Therefore, at this moment the method of synthesis by direct thermal condensation is best for us.

4.2.3 Microwave-assisted synthesis



Scheme 4.3 Coumaroyl tyramine derivatives synthesis by using microwave irradiation.

Due to the high yield from Table 4.2 entry **1** and **2**, we applied the similar condition using microwave irradiation. From the result in Table 4.3, the conditions using 1mol% boric acid (entry **6**) provided a very low yield compared to the conventional condition. Surprisingly, microwave synthesis using the cyanuric chloride as coupling reagents generated amide **49b** in high yield when compared with condition in entry **2** from Table 4.2, which occurred at room temperature.

Table 4.3 The optimization for the synthesis of amide **49b** using microwave irradiation.

Entry	Carboxylic acid (eq)	Amine (eq)	Coupling reagent	Solvent	Time	%Yield
1	1	4	1 mol% $B(OH)_3$	toluene	1h	5
2	2	2	Cyanuric chloride (1eq), NEt_3 (4eq)	DCM	6min	89

The obstacle of reaction in a liquid state when heated in a microwave oven is explosion and lost reactants and product when the solvent reached a boiling point. We solved the issue by using silica gel as a base to bind the reactants and changed from liquid state to solid state by removing the solvent with evaporation.

4.2.4 Microwave-assisted synthesis with silica gel.

39a **40** **41a**

Scheme 4.4 Coumaroyl tyramine derivatives synthesis by using microwave-assisted synthesis with silica gel.

Table 4.4 shows that, in entry **1** the reaction is not using coupling reagent and entry **2** the reaction is using boric acid as a coupling reagent which got a high yield in 60% and 52 % respectively. The other reaction used cyanuric chloride in a presence

of NEt_3 and DMAP, DCC and HOBt as a coupling reagent could result in a low yield. However, they were not dissolved and non-homogenous with silica gel.

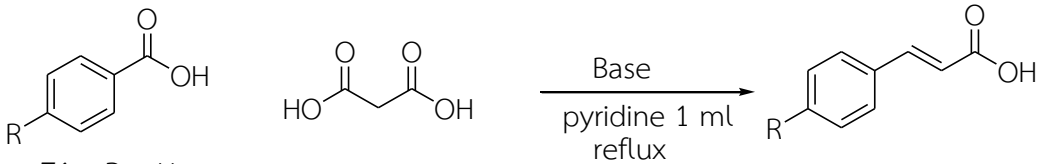
Table 4.4 The optimization for the synthesis of amide **41a** using MWI with silica gel.

Entry	Carboxylic acid (eq)	Amine (eq)	Coupling reagent	Solvent	Time	%Yield
1	1	1.2	-	EA	1h	60
2	1	4	1 mol% $B(OH)_3$	EA	1h	52
3	2	2	Cyanuric chloride (1eq), NEt_3(4eq)	DCM	6min	36
4	1	1.2	DMAP/DCC	DCM	1h	-
5	1	1.2	DMAP/DCC/HOBT	DCM	1h	34

4.3 Cinnamic acid synthesis

Some cinnamic acid derivatives were not available in our lab and it typically takes about 2-3 weeks to order the chemicals in Thailand. Therefore, we then decided to synthesize our own cinnamic acid derivatives. We began with the simple well-known Doebner modification condition. The attempts on the condition under microwave irradiation were also studied. Furthermore, we further investigated combining the acid synthesis with the amide synthesis to create a one-pot synthetic procedure.

4.3.1 Cinnamic acid synthesis by reflux



71a: R = H
71b: R = OCH_3

39a: R = H
39b: R = OCH_3

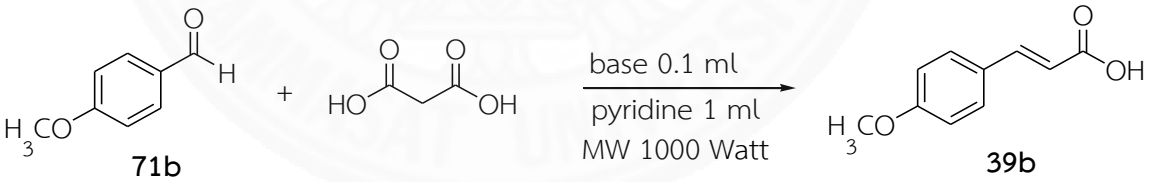
Scheme 4.5 Synthesis of cinnamic acid derivatives by Doebner modification condition

We first investigated to find the optimum conditions for the synthesis of cinnamic acid derivatives as shown in Table 4.5 The base and solvent were varied. We found that the conditions using piperidine (entry **7**) as a base generated *p*-methoxy cinnamic acid with the highest yield.

Table 4.5 The optimization condition for cinnamic acid synthesis.

Entry	Carboxylic acid (eq)	Malonic acid (eq)	base	Solvent	Temp. (℃)	Time(h)	Yield%
1	1	1.2	NH_4OAc 10 eq	H_2O	100	6	40
2	1	1.2	NH_4OAc 10 eq	NEt_3 0.45mL	90	53	65
3	1	1.2	NH_4OAc 10 eq	Pyridine	115	24	62
4	1	1.2	NH_4OH 35%	NEt_3 0.45mL	90	46	66
5	1	1.2	NH_4OH 35%	Pyridine	115	46	51-80
6	1	1.2	-	Pyridine	115	4 Days	29
7	1	4	Piperidine 0.1 mL	Pyridine	115	24	93

4.3.2 Cinnamic acid synthesis under microwave irradiation



Scheme 4.6 Synthesis of *p*-methoxy cinnamic acid under microwave irradiation.

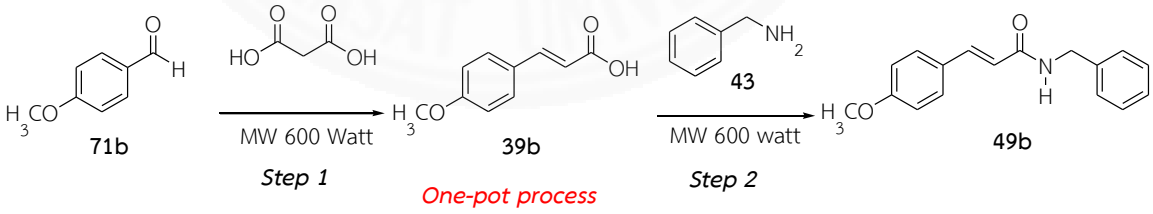
We then applied the condition from previous section (Table 4.5, entry **7**) to react under microwave irradiation in a domestic microwave oven. The reactions (Table 4.5, entry **5**) were completed within 3 min 10 sec with the improved yields over conventional heating method. We then investigated to find the optimum amount of malonic acid. The results showed that the yield decreased with the reduction of malonic acid (Table 4.6, entry **2-4**).

Table 4.6 The optimization for the synthesis of *p*-methoxy cinnamic acid by microwave irradiation.

Entry	Aldehyde (eq)	Malonic acid (eq)	Base	Solvent	Time(min)	Yield%
1	1	4	NH_4OH 35%	Pyridine	51	11
2	1	1	Piperidine	Pyridine	13.20	23
3	1	3	Piperidine	Pyridine	26.5	71
4	1	3.5	Piperidine	Pyridine	10	95
5	1	4	Piperidine	Pyridine	3.10	96

4.4. Two-step, one-pot strategy

As seen from the above results, the synthesis of cinnamic acids and amide formation could be done under the microwave irradiation. We then hypothesized that these two steps could be able to be combined into one step without the purification of the acid synthesis step. Therefore, the two-steps one-pot strategy was investigated as shown in Table 4.7. The results showed that, we optimized the first step by sodium acetate in acetic acid, ammonium acetic in pyridine, and piperidine in pyridine and the second step was optimized by 1 mol % boric acid and cyanuric chloride in the present of triethylamine, but we still got a low yield.



Scheme 4.7 Two-step, one-pot strategy.

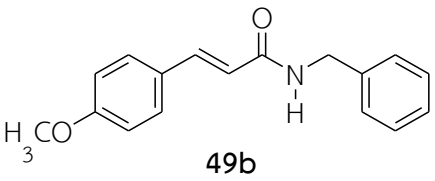
In entry **9**, we still used piperidine and pyridine as a reagent and solvent respectively because they could get high yield in first step and optimized the second step by using silica gel support without coupling reagent that generated the product in high yield. This reaction got a high yield because there is no explosion and loss of product.

Table 4.7 The optimization for one-pot process.

Entry	Step 1			Step 2			Yield%
	Reagent	Solvent	Time	Reagent	Solvent	Time	
1	NaOAc 0.5 eq	AcOH 2 eq	20 min	1 mol % $B(OH)_3$	Toluene	1 h	-
2	NaOAc 0.5 eq	AcOH 2 eq	20 min	Cyanuric chloride (1 eq), NEt_3 (4 eq)	DCM	3 min	5.4-53
3	NH_4COOCH_3 0.5 eq	Pyridine 1 mL	1.40min	Cyanuric chloride (1 eq), NEt_3 (4 eq)	DCM	4.10 min	3
4	NH_4COOCH_3 0.5 eq	Pyridine 1 mL	1.40min	Cyanuric chloride (1 eq), NEt_3 (4 eq)	-	4.10 min	3.1
5	Piperidine 0.1 mL	Pyridine 1 mL	5 min	1% $B(OH)_3$	Toluene	1 h	trace
6	Piperidine 0.1 mL	Pyridine 1 mL	5 min	Cyanuric chloride (1 eq), NEt_3 (4 eq)	DCM	3 min	37
7	Piperidine 0.1 mL	Pyridine 1 mL	3 min	Cyanuric chloride (1 eq), NEt_3 (4 eq)	-	6 min	No product
8	Piperidine 0.1 mL	Pyridine 1 mL	3 min	-	-	10 min	No product
9	Piperidine 0.1 mL	Pyridine 1 mL	3 min	Silica gel support	EA	60 min	83

According to the results obtained, a simple and efficient synthetic method for the synthesis of coumaroyl tyramine derivatives is two-step, one-pot strategy under the microwave irradiation, which synthesize amide from aldehyde and malonic acid as starting reactant and using piperidine and pyridine as reagent in first step to generated several cinnamic acid without purification, and then react with amine by using silica gel for changed from liquid state to solid state. This method can give the amide in high yield and reduce time of reaction.

4.5 Characterization of the *N*-benzyl-4-methoxycinnamamide (49b)



49b

N-benzyl-4-methoxycinnamamide

Figure 4.3 Structure of *N*-benzyl-4-methoxycinnamamide (**49b**).

The *N*-benzyl-4-methoxycinnamamide (**49b,** Figure 4.3) was characterized by ^{1}H NMR and FTIR, which are in good agreement with the literature data,[42] as follow:

^{1}H-NMR (400MHz, $CDCl_3$) **δ**(ppm) 7.38-7.19 (m, 4H, Ar-H), 6.83-6.80 (m, 5H, Ar-H), 6.25-6.19 (d, 1H, =C-H, *J* = 22.85 Hz), 4.51-4.49 (d, 1H, =C-H, *J* = 13.86 Hz), 3.89 (s, 3H, -OCH_3), 3.76-3.74 (d, 2H, CH_2, *J* = 10.36 Hz)

^{1}H-NMR spectrum showed the signals of 9 aromatic at position **δ** 7.38-6.80 ppm. The spectrum also showed the significant of vinylic proton at **δ** 6.25-6.19 (d, *J* = 22.85 Hz, 1H) and 4.51-4.49 (d, *J* = 13.86 Hz, 1H). Moreover, the spectrum showed the significant of methoxy group at **δ** 3.89 ppm as shown in Figure 4.4

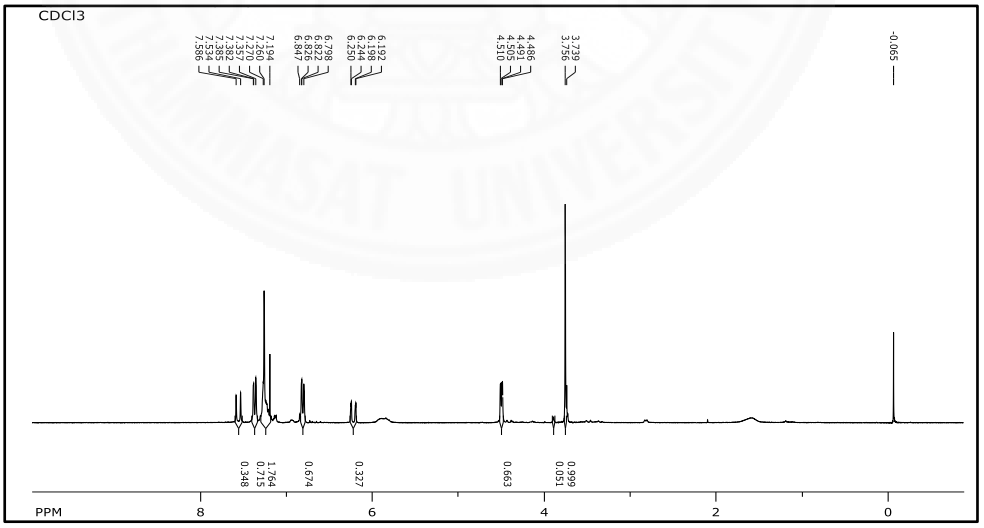


Figure 4.4 ^{1}H-NMR of *N*-benzyl-4-methoxycinnamamide (**49b**) in $CDCl_3$.

FTIR spectrum (Figure 4.5) presented a broad peak of N-H stretching in the region between 3000 and 3600 cm^{-1}. Moreover, The C=O stretching modes presented

between 1612 and 1491 cm^{-1} of amide. The C=C stretching will appear as a sharp peak between 1500 and 1600 cm^{-1}. The sharp peaks belong to aromatic and alkene functional groups. The IR spectrum data of *N*-benzyl-4-methoxycinnamamide (**49b**) were indicated in Table 4.8.

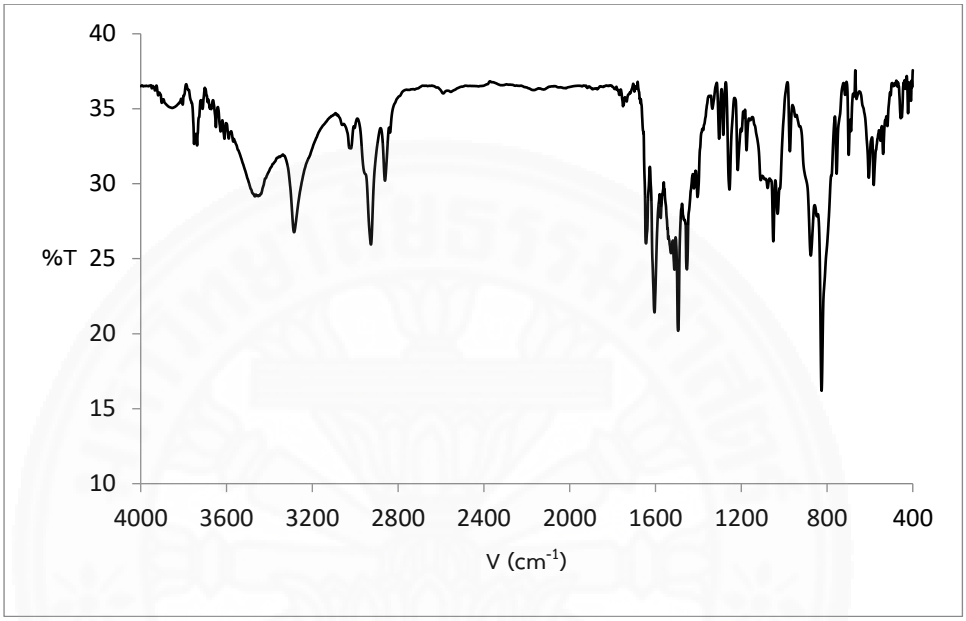


Figure 4.5 FTIR of *N*-benzyl-4-methoxycinnamamide **(49b).**

Table 4.8. FTIR spectrum data of *N*-benzyl-4-methoxycinnamamide **(49b).**

Entry	Vibration frequency (cm^{-1})	Assignment
1	3000-3600 (br.)	N-H stretching
2	3076, 3031	=C-H stretching
3	1608 (s)	C=O stretching
4	1515	N-H bending
5	1589, 1534	C=C stretching
6	1491	C-N stretching

Mass spectrum of *N*-benzyl-4-methoxycinnamamide **(49b)** displayed a molecular ion peak at 268.8 m/z [M^{+1}].

4.6 Biological activity

From all of method, we synthesized 24 derivatives to study biological activities of the synthesized compounds as phytotoxicity, insecticidal activity, antifeedant activity, antioxidant activity, and antipancreatic lipase activity.

4.6.1 Phytotoxicity of lettuce seeds

This research study the effect of synthesized compounds to inhibit seed germination of lettuce seeds.

Table 4.9 The effect of coumaroyl tyramine derivatives to length of leaves, haulm, roots, and weight of lettuce seeds.

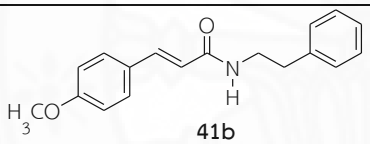
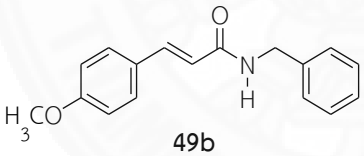
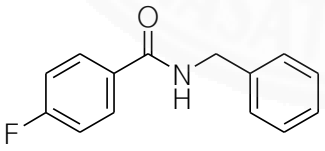
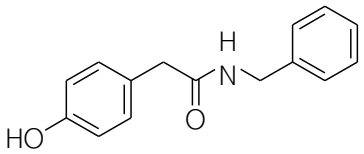
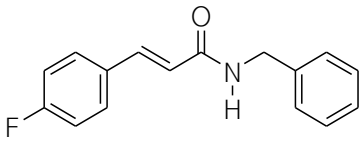
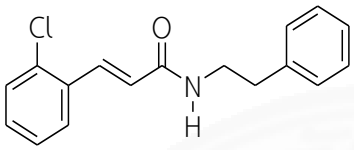
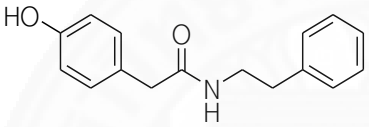
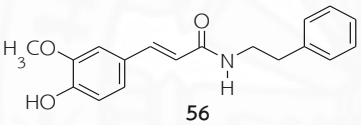
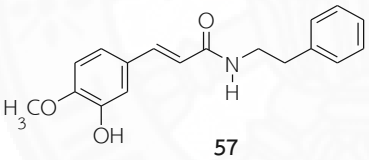
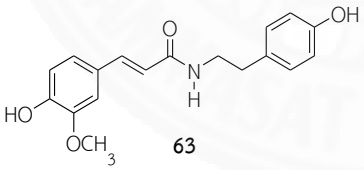
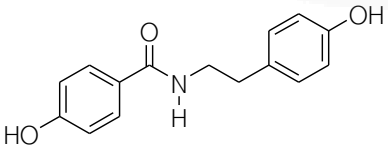
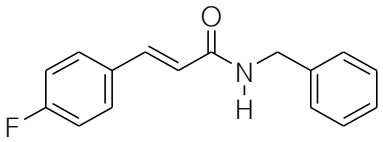
Entry	compound	Concentration (μg/mL)	Average of length (mm)			Weight (g)
			Roots	Haulm	Leaves	
1	 **41b**	500	40.17	2.37	10.90	100.6
		250	38.66	2.69	10.62	87.83
2	 **49b**	500	15.57	2.80	10.93	110.5
		250	17.63	2.80	10.8	108.7
3	 **50**	500	17.72	2.53	9.37	80.13
		250	16.5	2.83	8.93	68.97
4	 **51**	500	4.97	2.48	10.05	75.33
		250	5.13	3.42	9.09	82.30

Table 4.9 The effect of coumaroyl tyramine derivatives to length of leaves, haulm, roots, and weight of lettuce seeds. (continue)

5	 **52**	**500**	**3.07**	**2.10**	**8.83**	86.57
		250	**3.11**	**1.85**	**9.81**	92.30
6	 **54**	500	37.63	3.39	11.70	115.0
		250	32.27	3.00	11.93	107.7
7	 **55**	500	13.23	3.71	11.80	126.8
		250	23.23	3.67	12.30	124.1
8	 **56**	500	33.97	4.32	12.27	103.5
		250	30.8	4.60	13.17	116.2
9	 **57**	500	11.4	3.03	12.53	114.5
		250	10.99	3.03	11.51	112.8
10	 **63**	500	18.9	4.29	11.17	105.8
		250	29.3	5.67	12.27	106.6
11	 **64**	500	9.53	3.15	11.03	105.0
		250	12.7	5.07	11.9	96.03
12	Acetone	-	35	4.8	12.5	124.8

The results of phytotoxicity in Table 4.9 show that compound **52**, entry **4** which have fluorine substitute at cinnamic acid side at concentration 250 and 500 μg/mL had

affected lettuce seeds growth when compare with acetone as a control. Therefore, we decreased the concentration of the solution of compound **52**.



52

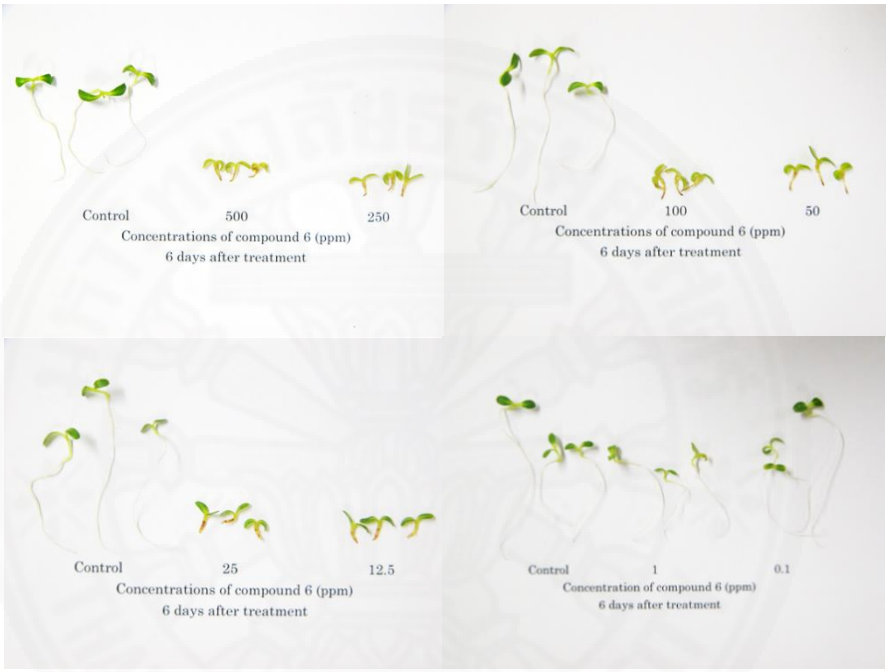
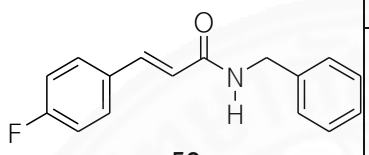


Figure 4.6 Growth of lettuce seeds containing compound **52** at concentration 500, 250, 100, 50, 25, 12.5, 1, and 0.1 μg/mL.

From Figure 4.3 and Table 4.10 show that the compound **52** at concentration 12.5, 25, 50, 100, 250 and 500 μg/mL have the potential to cause plant diseases because they can inhibit the growth of leaves, haulm, roots, and weight of lettuce seeds

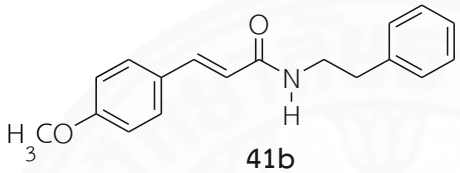
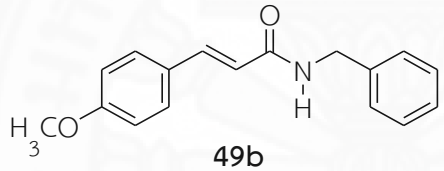
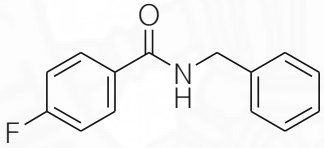
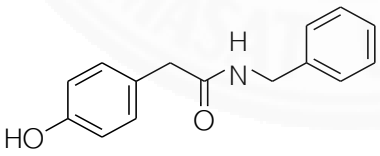
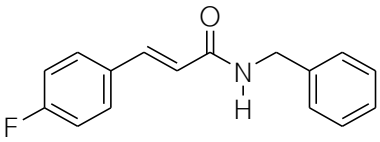
Table 4.10 The effect of compound **52** to length of leaves, haulm, roots, and weight of lettuce seeds.

Entry	compound	Concentration (μg/mL)	Average of length (mm)			Weight (g)
			Roots	Haulm	Leaves	
1	 **52**	500	3.07	2.10	8.83	86.57
		250	3.11	1.85	9.81	92.30
		100	4.57	1.53	8.80	90.33
		50	4.33	1.87	9.20	108.6
		25	3.00	1.70	9.83	106.3
		12.5	4.29	1.69	9.93	109.9
		1	35.17	3.27	10.53	114.6
		0.1	42.57	4.07	11.67	110.6
2	Acetone	-	35	4.8	12.5	124.8

4.6.2 Insecticidal assay of common cutworms

From the table 4.11 shown that compound **49b** and **50** were active against the survival of the larvae of common cutworm, *Spodopteralitura* because 6 hours after the experiment showed that there were less than half of the total number of live worms. Therefore, compounds **49b** and **50** have insecticidal activity.

Table 4.11 The effect of coumaroyl tyramine derivatives to Insecticide the larvae of common cutworm.

Entry	Compounds	Number of live worms						Results
		n=1	n=2	n=3	n=4	n=5	Average	
1	Control	4	4	5	4	3	4	-
2	 **41b**	3	4	4	2	2	3	-
3	 **49b**	3	2	2	4	3	2.8	+
4	 **50**	2	3	2	4	3	2.8	+
5	 **51**	2	3	4	4	4	3.4	-
6	 **52**	3	4	5	4	2	3.6	-

4.6.3 Antifeedant activity

Table 4.12 shows the antifeedant index (AFI) of the larvae of common cutworm, *Spodopteralitura* with coumaroyl tyramine derivatives coated on sweet potatoes. The results indicate that most of all cinnamic acid derived compounds did not have antifeedant activity as AFIs are higher 40.

Table 4.12 The effect of coumaroyl tyramine derivatives to area of sweet potato leaves before and after they were eaten with worms and antifeedant index (AFI).

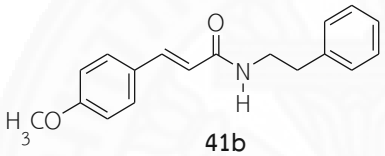
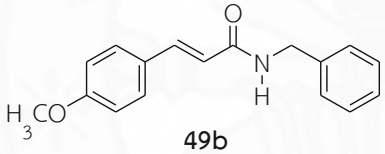
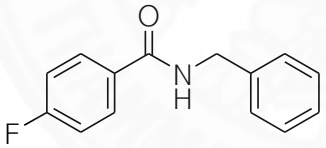
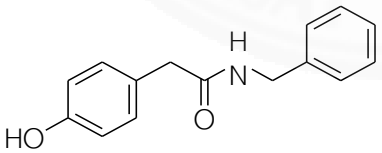
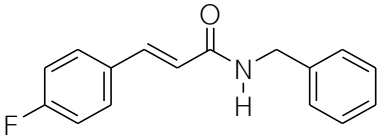
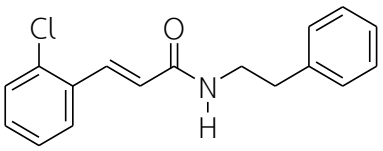
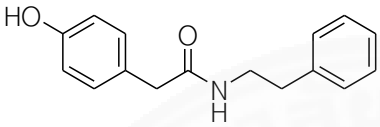
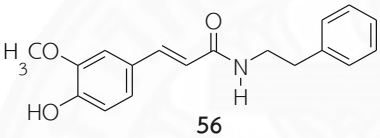
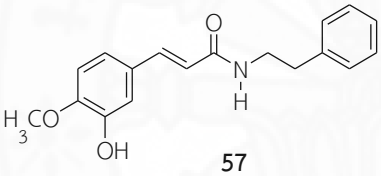
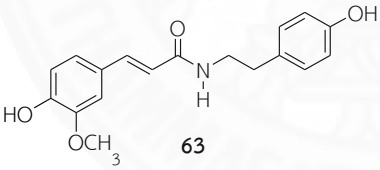
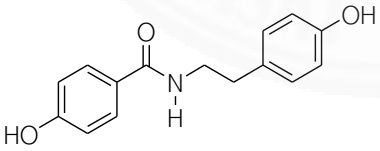
Entry	compound	n	Area (pixl)		Antifeedant index (AFI)
			Treatment area	Control area	
1	 **41b**	1	20120	21288	44.64 ± 13.91
		2	14374	17964	
		3	27834	9666	
2	 **49b**	1	33793	10607	24.10 ± 6.81
		2	33614	19572	
		3	34496	27778	
3	 **50**	1	32510	24185	34.61 ± 10.69
		2	32234	31003	
		3	32902	19128	
4	 **51**	1	38870	25970	21.00 ± 15.28
		2	33490	10332	
		3	28048	20132	
5	 **52**	1	20826	21138	52.50 ± 2.73
		2	14318	19481	
		3	16139	17526	

Table 4.12 The effect of coumaroyl tyramine derivatives to area of sweet potato leaves before and after they were eaten with worms and antifeedant index (AFI). (continue)

6	 **54**	1	31697	13884	32.99 ± 12.54
		2	25991	20542	
		3	17410	23686	
7	 **55**	1	18937	13260	43.71 ± 0.49
		2	9373	0	
		3	24702	21346	
8	 **56**	1	9422	21739	60.87 ± 2.46
		2	5379	12835	
		3	0	0	
9	 **57**	1	22974	22686	53.68 ± 5.79
		2	5124	14507	
		3	2681	2482	
10	 **63**	1	2989	4312	50.56 ± 0.49
		2	5767	6066	
		3	8033	16145	
11	 **64**	1	23218	11308	36.06 ± 1.20
		2	21214	5430	
		3	8440	0	

This results mean that they all show no activity at concentration 0.5 mg/dish excepted compound **49b**, *N*-benzyl-4-methoxycinnamamide and compound **54**, 2-chloro-*N*-(2-phenylethyl)prop-2-enamide had moderate antifeedant activity at 24.10 ± 6.81 and 32.99 ± 12.54 of AFI respectively.

In addition, compounds **50** and **64** which had benzoic acid and compound **51** which had 4-hydroxyphenylacetic acid and benzylamine of three compounds had moderate antifeedant activity at 34.61 ± 10.69, 36.06 ± 1.20 and 21.00 ± 15.28 of AFI, respectively.

4.6.4 Determination of free radical scavenging activity

Antioxidant activity of the synthesized compounds were evaluated with the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay.[41] Gallic acid was used as a positive control.

Table 4.13 Antioxidant activity of coumaroyl tyramine derivatives.

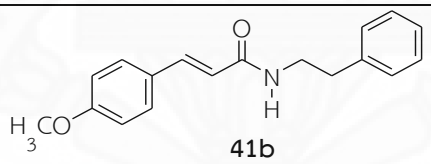
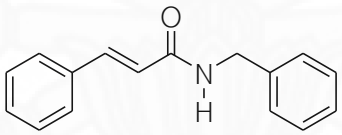
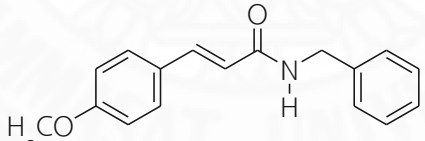
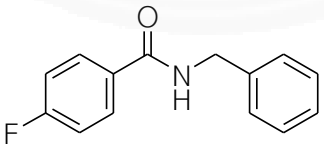
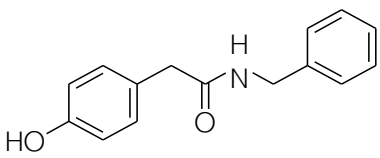
Entry	Compounds	IC_{50}(μg/mL)
1	 **41b**	>1000
2	 **44a**	>1000
3	 **49b**	>1000
4	 **50**	>1000
5	 **51**	**>1000**

Table 4.13 Antioxidant activity of coumaroyl tyramine derivatives. (continue)

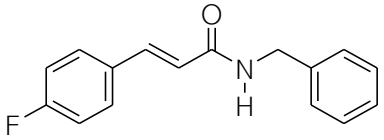
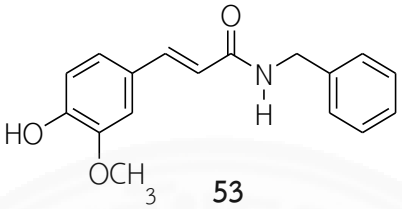
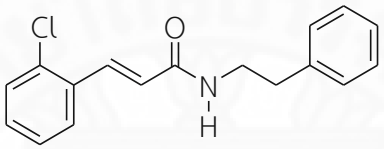
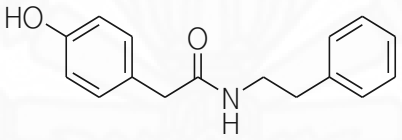
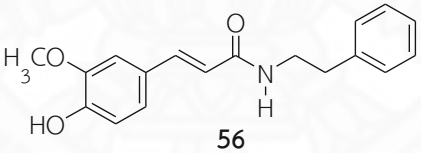
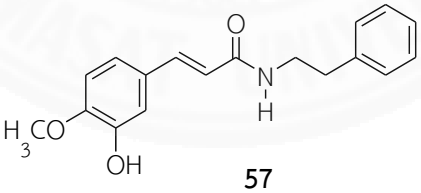
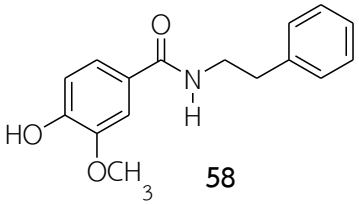
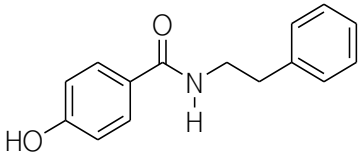
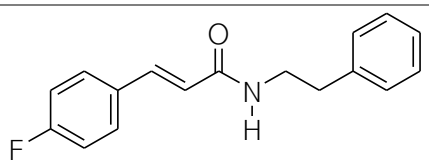
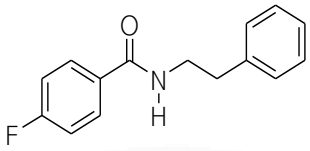
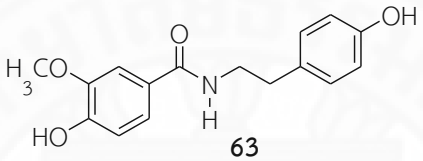
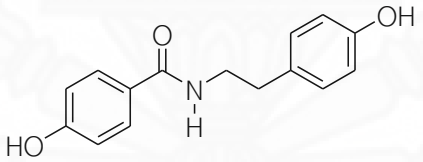
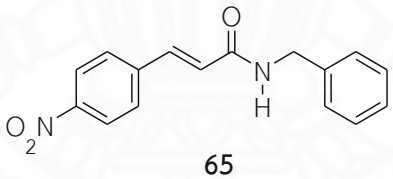
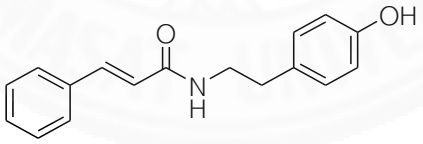
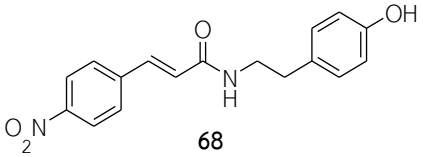
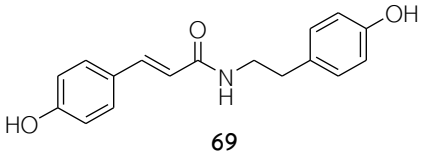
6	 **52**	>1000
7	 **53**	218.32 ± 1.08
8	 **54**	>1000
9	 **55**	>1000
10	 **56**	42.46 ± 1.52
11	 **57**	202 ± 0.80
12	 **58**	>1000
13	 **59**	>1000

Table 4.13 Antioxidant activity of coumaroyl tyramine derivatives. (continue)

14	 **60**	>1000
15	 **61**	>1000
16	 **63**	38.74
17	 **64**	>1000
18	 **65**	>1000
19	 **66**	>1000
20	 **68**	>1000
21	 **69**	>1000
22	Gallic acid	3.68

The result shown in Table 4.13 indicated that *trans-N*-coumaroyl tyramine (**69**) no activity at concentration 1000 µg/mL as reported a literature survey.[11] According to a literature survey,[42] compound containing **53**, **56**, **57**, and **63** which had hydroxyl and methoxy group at position 3 or 4 in cinnamic acid side showed moderate antioxidant inhibition. While other compounds contain only the substituent on position 4 showed low or no activity.

When compared the compound **53** and **56** which have same cinnamic side but different amine, found the compound **56** high activity when have more carbon atom in amine side. While the position of methoxy and hydroxy at 3 and 4 position of the compound **56** have high activity more than the compound **57** which have position of hydroxy and methoxy at 3 and 4 position. Moreover, the hydroxy at 4 position of amine (**63**) improve activity more than no substituent (**50**) as shown in Figure 4.7.

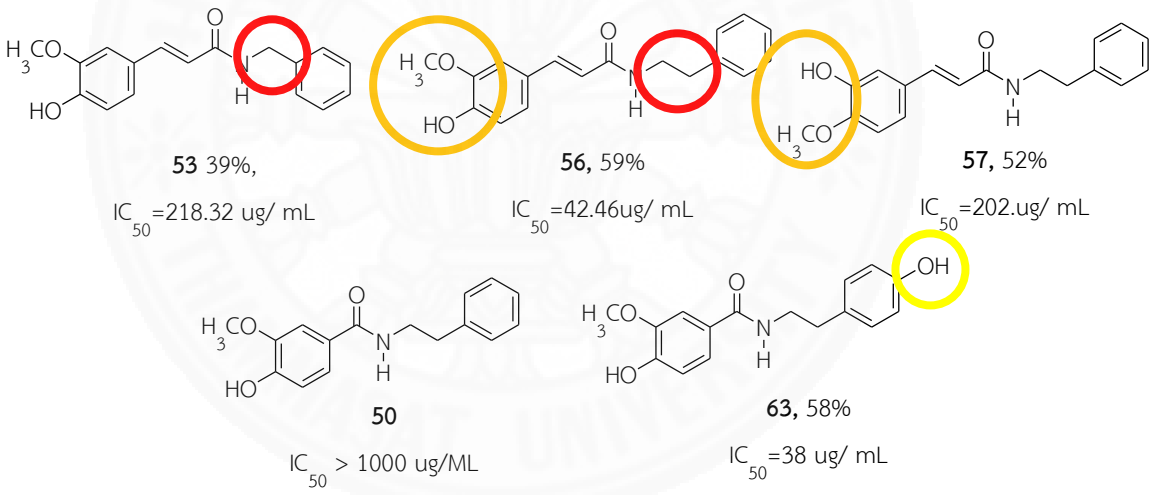


Figure 4.7. The structure of compound **50**, **53**, **56**, **57**, and **63**

In addition, it must be noted that the presence of hydroxy group on either position 3 or 4 resulted in about 50 times lower IC_{50} values. Also, the chain length on the amine part may be important.

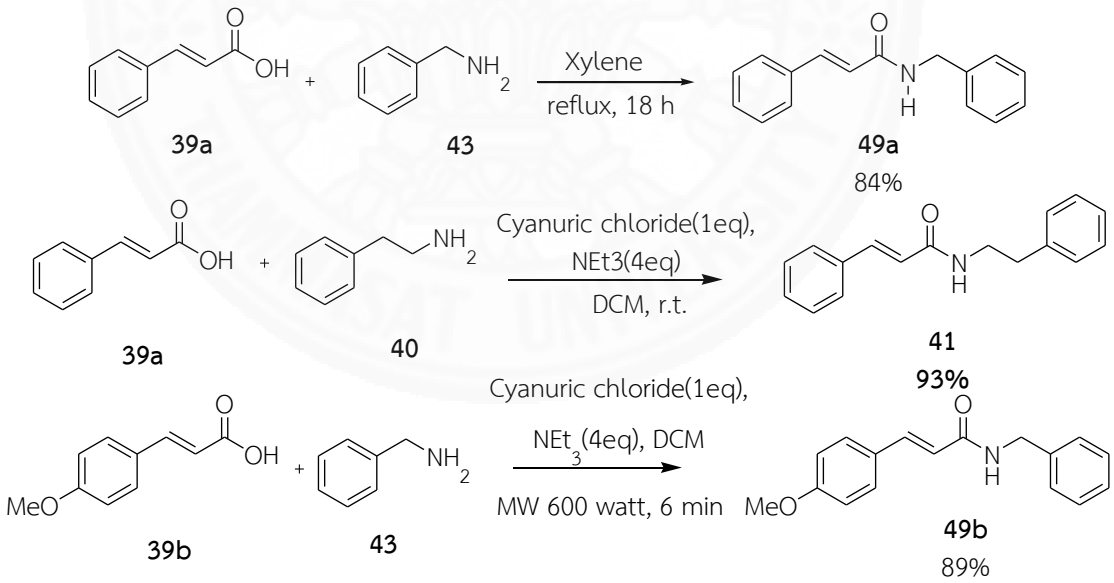
4.6.5 Determination of antipancreatic lipase activity

As a result of both processes, it can be concluded that the lipase enzyme was inactive because in the reaction using orlistat as a positive control, the lipase color remained yellow and the absorbency was close to the controls that are without lipase.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Synthesis section

trans-N-coumaroyltyramine is a natural compound isolated from *Nelumbo nucifera* that shows potent biological activity as an antioxidant and has an anti-obesity capacity by inhibiting pancreatic lipase and adipocyte differentiation. We have reported here a simple and efficient synthetic method to synthesize coumaroyl tyramine derivatives by using dean-stark between carboxylic acid and amine in xylene yielding 39-86%. The synthesis of amides also worked using cyanuric chloride as coupling reagent in 76-79% yield and microwave synthesis by using cyanuric chloride as a coupling reagent yielding 89% within 6 minutes. Upon those methods, we have synthesized 24 derivatives of coumaroyl tyramine derivatives.



Scheme 5.1. The conditions to synthesize coumaroyl tyramine derivatives (a) reflux in xylene as a solvent, (b) cyanuric chloride as coupling reagent at room temperature, and (c) cyanuric chloride as coupling reagent at microwave synthesis

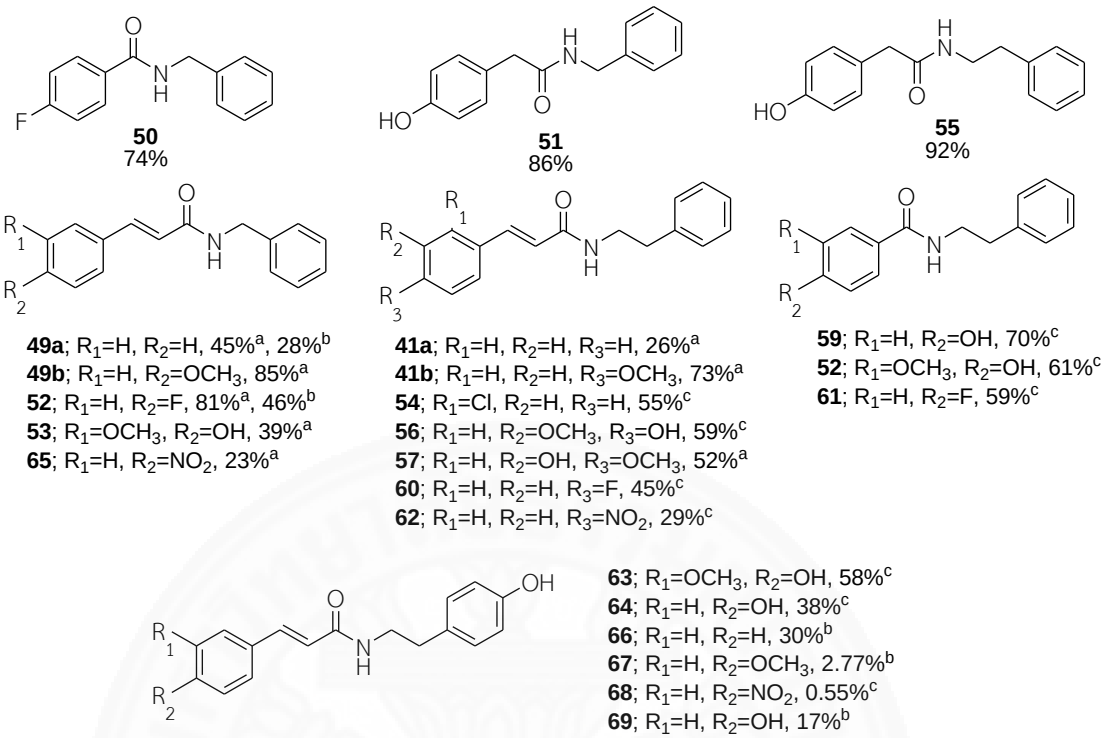
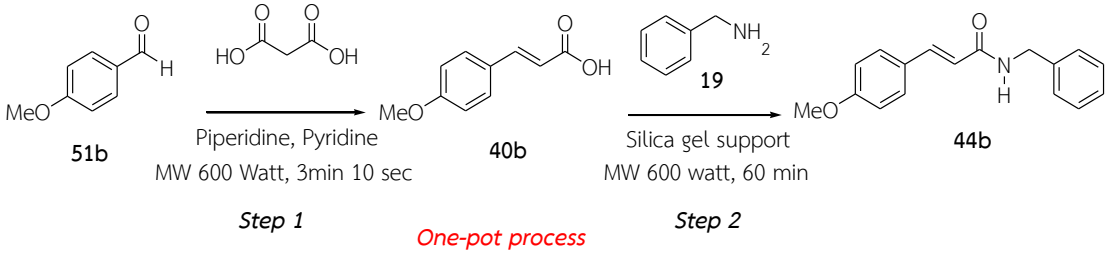


Figure 5.1. 24 structures of coumaroyl tyramine derivatives; (a) reflux in xylene as a solvent, (b) cyanuric chloride as coupling reagent at room temperature, and (c) diisopropylethylamine as coupling reagent at aminolysis ester.

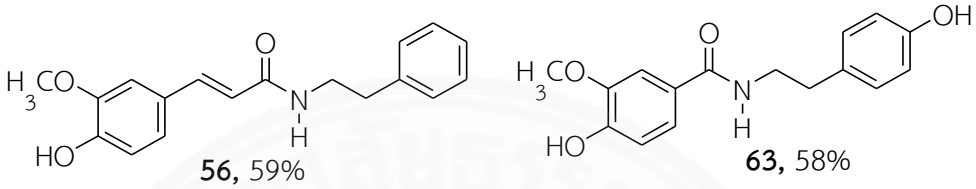
We found the simple and efficient synthetic method for the synthesis of coumaroyl tyramine derivatives is two-step, one-pot synthetic procedure for the direct conversion of aromatic aldehydes to aromatic amides by using piperidine and pyridine as a reagent and solvent respectively in first step and in the second step is using silica gel support without coupling reagent can generate the product in 83% yield. This procedure can be synthesized in a very short time and reduce the purification process of acid synthesis.



Scheme 5.2. The optimum conditions to synthesize coumaroyl tyramine derivatives by microwave-assisted synthesis.

5.2 Biological activity

For the antioxidant activity screening, we found that the substituent on the aromatic ring of carboxylic acid counterpart is significant for the antioxidant activity. The result showed that compounds **56** and **63** gave the highest antioxidant activity at IC_{50} values 42.46 and 38 µg/mL, respectively.



56, 59%

IC_{50}=42.46ug/ mL

63, 58%

IC_{50}=38 ug/ mL

Figure 5.2. The compound **56** and **63**, which gave the highest antioxidant activity.

In addition, a variety of coumaroyltyramine derivatives were designed, synthesized, and tested of biological assays including phytotoxicity, insecticidal activity, antifeedant activity. To evaluate phytotoxicity activity, the obtained fluorine compound **52** have the potential to delay growth of plant, which can be used in herbicides. To evaluate insecticidal activity, the compounds **49b** and **50** have insecticidal activity. Moreover, to evaluate antifeedant activity, compound **49b**, **50**, **51, 55**, and **64** have moderate antifeedant activity at 24.10 ± 6.81, 34.61 ± 10.69, 21.00 ± 15.28, 32.99± 12.54, and 36.06 ± 1.20 of AFI, respectively. Therefore, the compounds **49b** and **50** can be used for insecticide and insect antifeedant activity of the larvae of common cutworm, which to be useful in agriculture.

5.3 Outlook

The microwave optimized conditions will be applied to synthesize coumaroyltyramine derivatives and to study the effects of compounds on insecticide compared with current insecticides.

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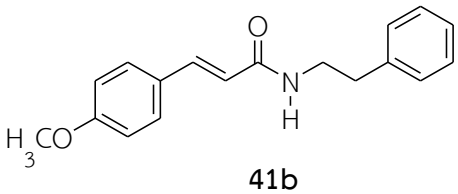
APPENDICES

APPENDICE A

Phytotoxicity of lettuce seeds



41b

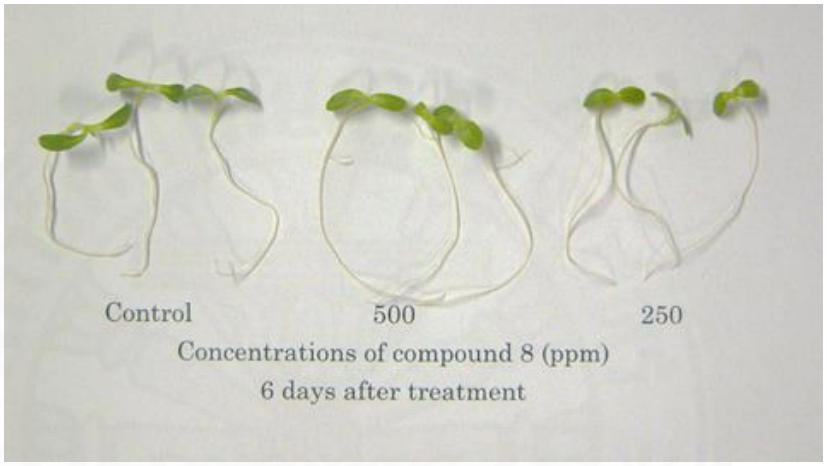
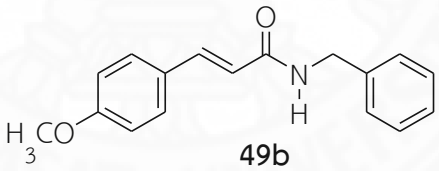
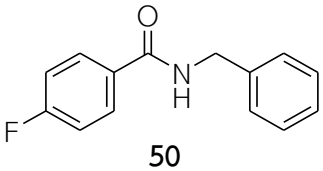


Figure A.1 Growth of lettuce seeds containing compound **41b** at concentration 500 and 250 µg/mL.



49b

Figure A.2 Growth of lettuce seeds containing compound **49b** at concentration 500 and 250 µg/mL.



50

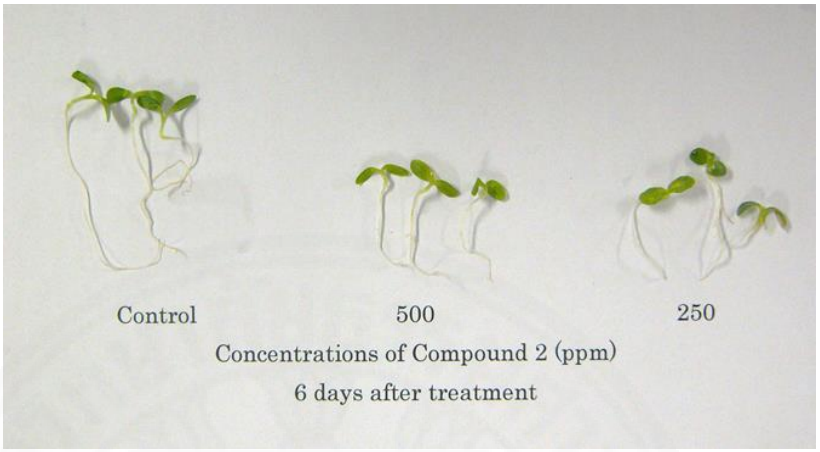
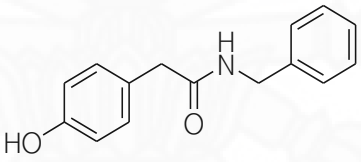


Figure A.3 Growth of lettuce seeds containing compound **50** at concentration 500 and 250 µg/mL.



51

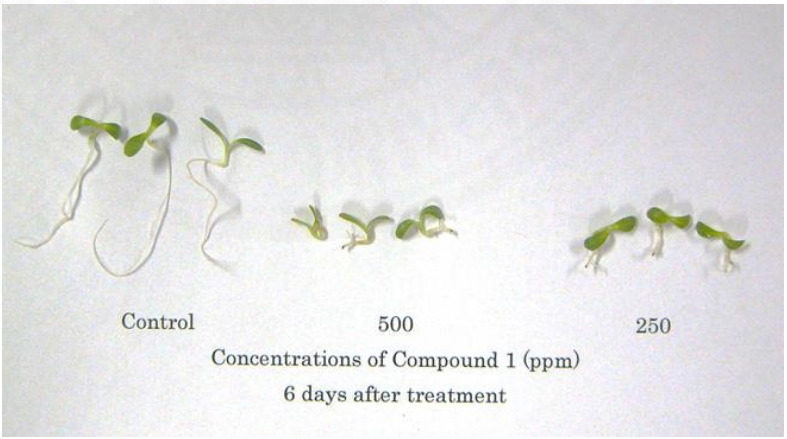
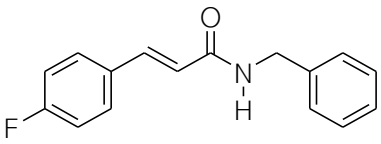


Figure A.4 Growth of lettuce seeds containing compound **51** at concentration 500 and 250 µg/mL.



52

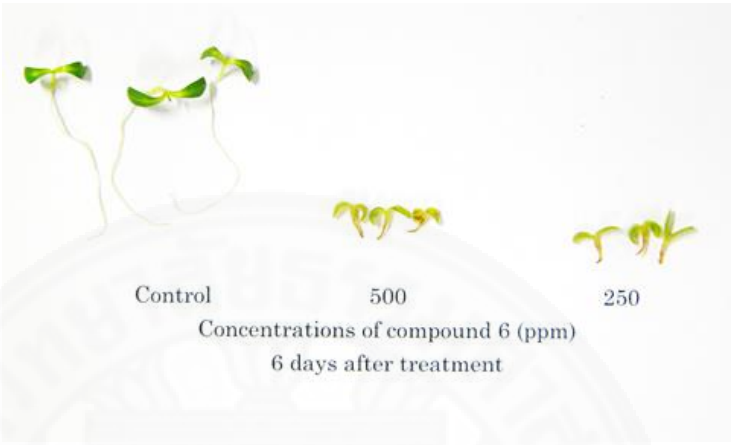
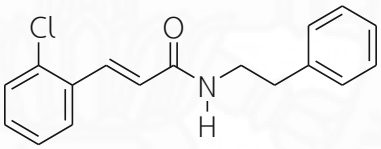


Figure A.5 Growth of lettuce seeds containing compound **52** at concentration 500 and 250 µg/mL.



54

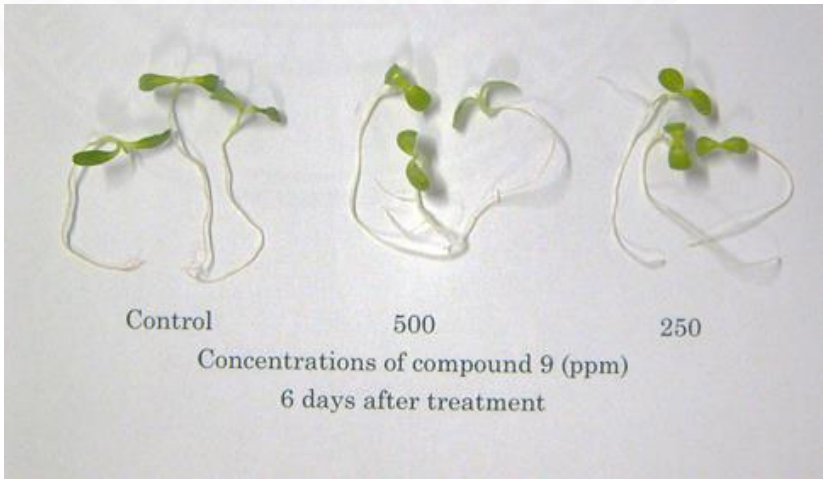
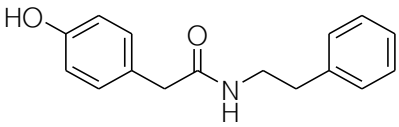
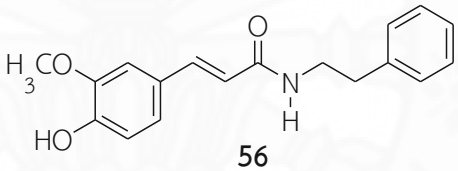


Figure A.6 Growth of lettuce seeds containing compound **54** at concentration 500 and 250 µg/mL.



55

Figure A.7 Growth of lettuce seeds containing compound **55** at concentration 500 and 250 µg/mL.



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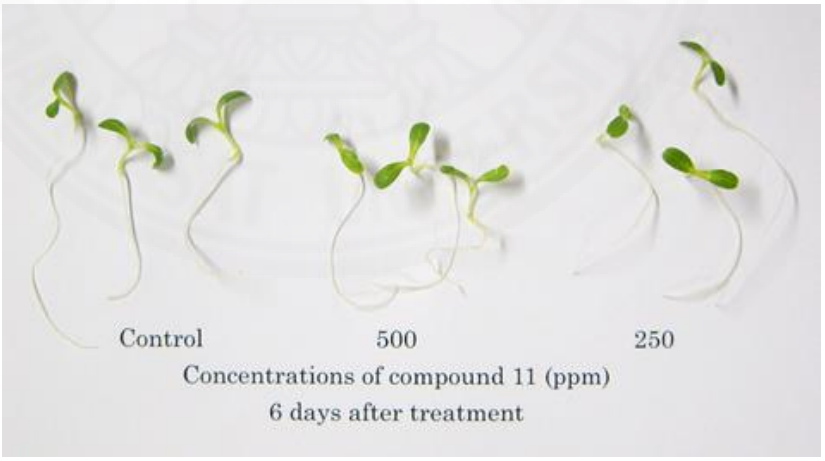
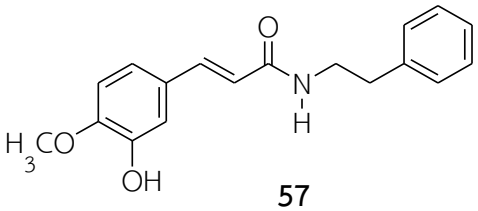
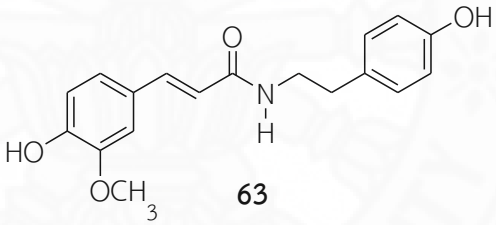


Figure A.8 Growth of lettuce seeds containing compound **56** at concentration 500 and 250 µg/mL.



57

Figure A.9 Growth of lettuce seeds containing compound **57** at concentration 500 and 250 µg/mL.



63

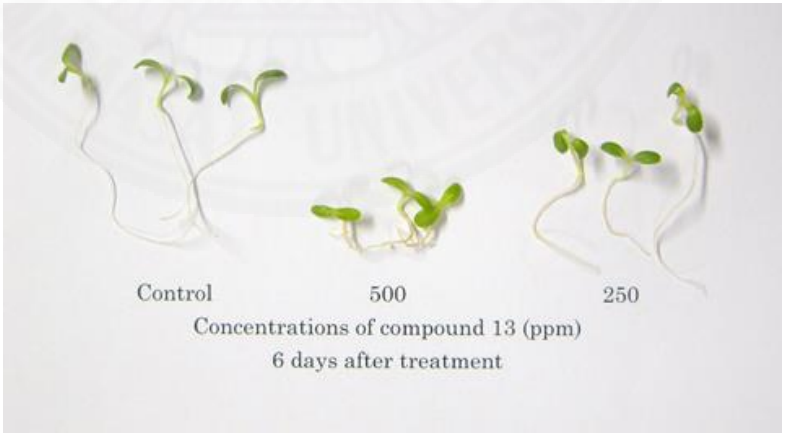
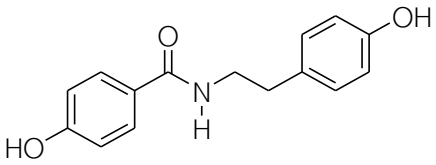


Figure A.10 Growth of lettuce seeds containing compound **63** at concentration 500 and 250 µg/mL.



64

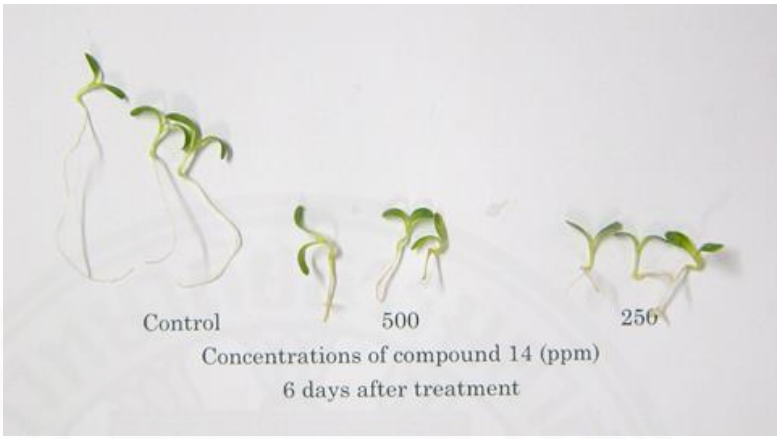


Figure A.11 Growth of lettuce seeds containing compound **64** at concentration 500 and 250 µg/mL.

APPENDICE B

Antifeedant activity

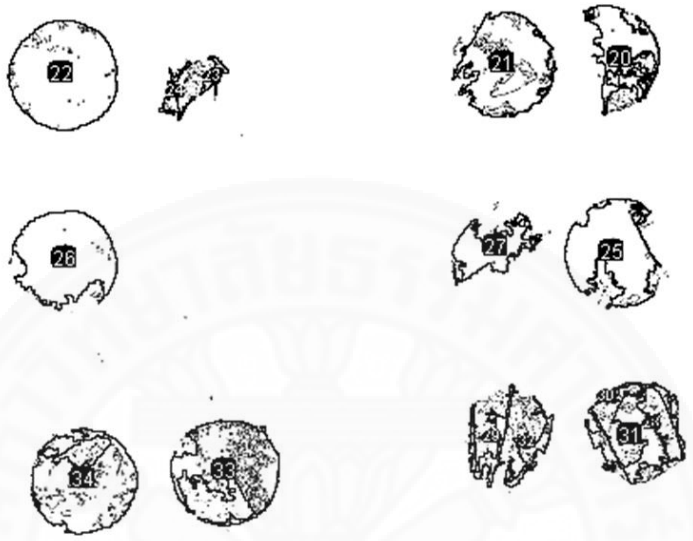


Figure B.1 The remaining areas of sweet potato leaves are coated with compound **41b** after being eaten by the worms.

Figure B.2 The remaining areas of sweet potato leaves are coated with compound **49b** after being eaten by the worms.

Figure B.3 The remaining areas of sweet potato leaves are coated with compound **50** after being eaten by the worms.

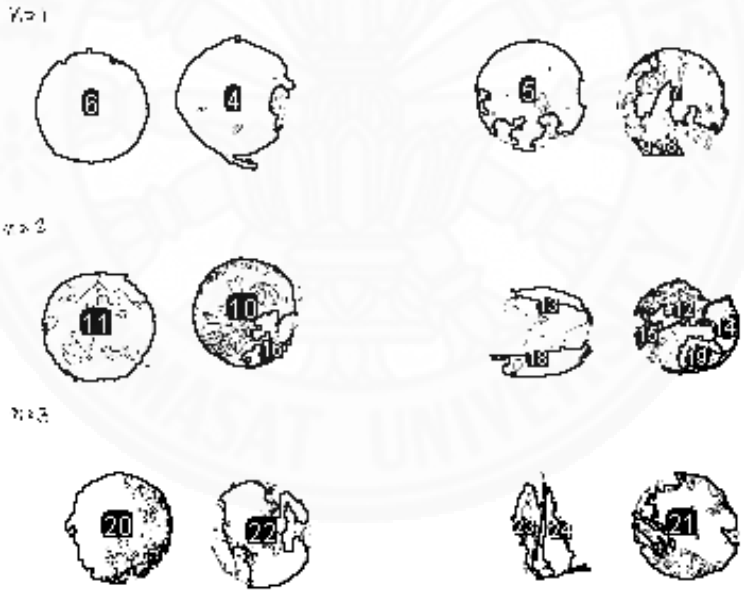


Figure B.4 The remaining areas of sweet potato leaves are coated with compound **51** after being eaten by the worms.

Figure B.5 The remaining areas of sweet potato leaves are coated with compound **52** after being eaten by the worms.

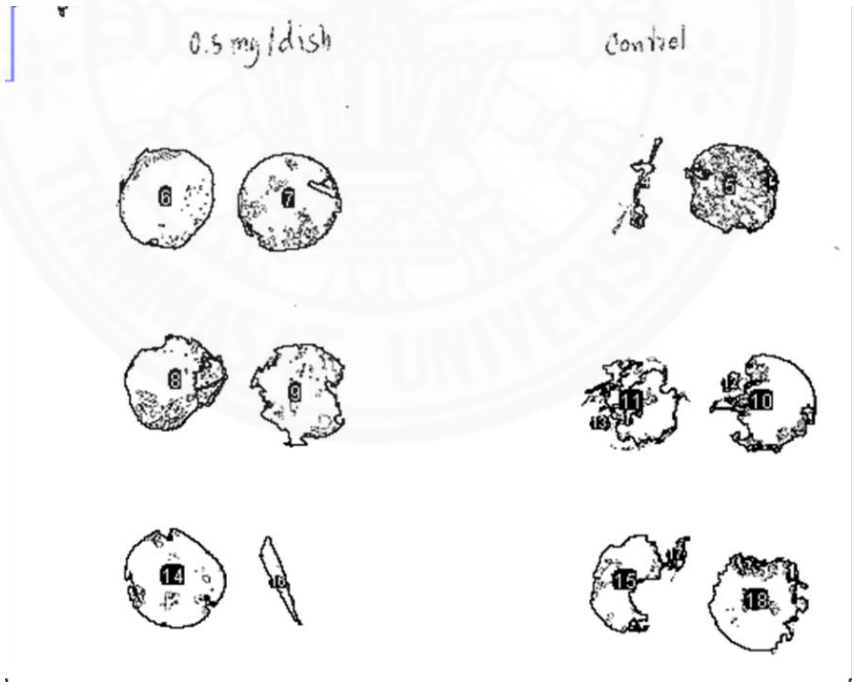


Figure B.6 The remaining areas of sweet potato leaves are coated with compound **54** after being eaten by the worms.

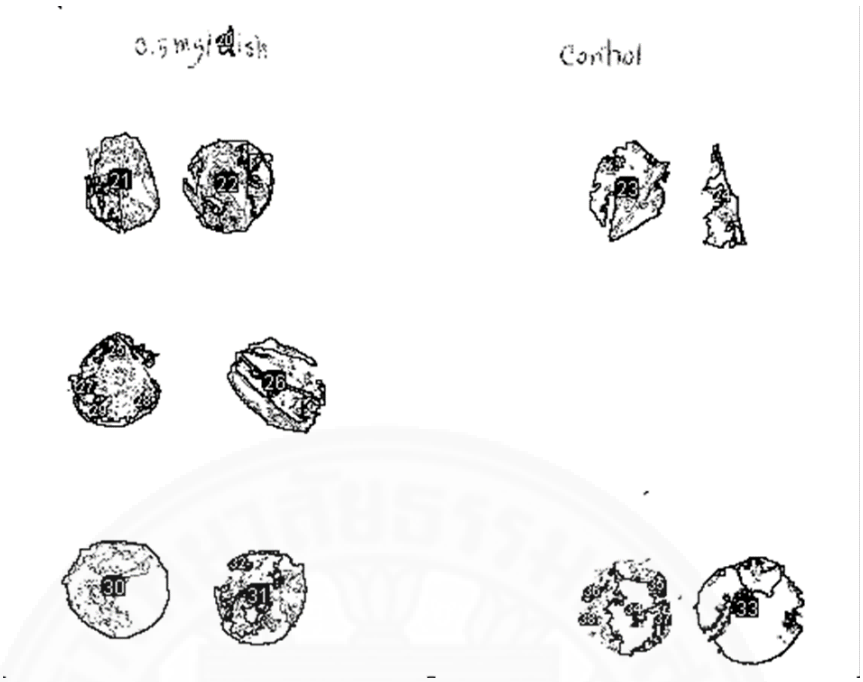


Figure B.7 The remaining areas of sweet potato leaves are coated with compound **55** after being eaten by the worms.

Figure B.8 The remaining areas of sweet potato leaves are coated with compound **56** after being eaten by the worms.

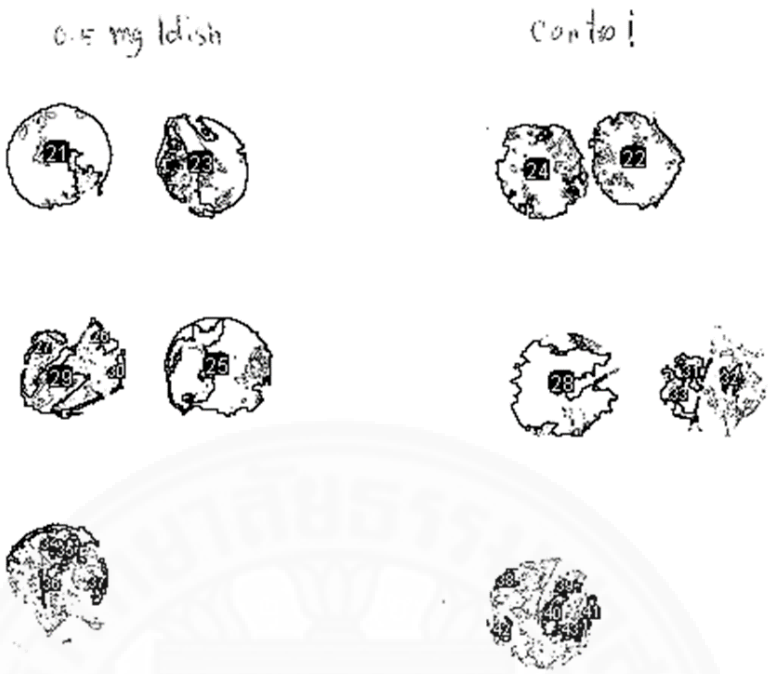


Figure B.9 The remaining areas of sweet potato leaves are coated with compound **57** after being eaten by the worms.

Figure B.10 The remaining areas of sweet potato leaves are coated with compound **63** after being eaten by the worms.

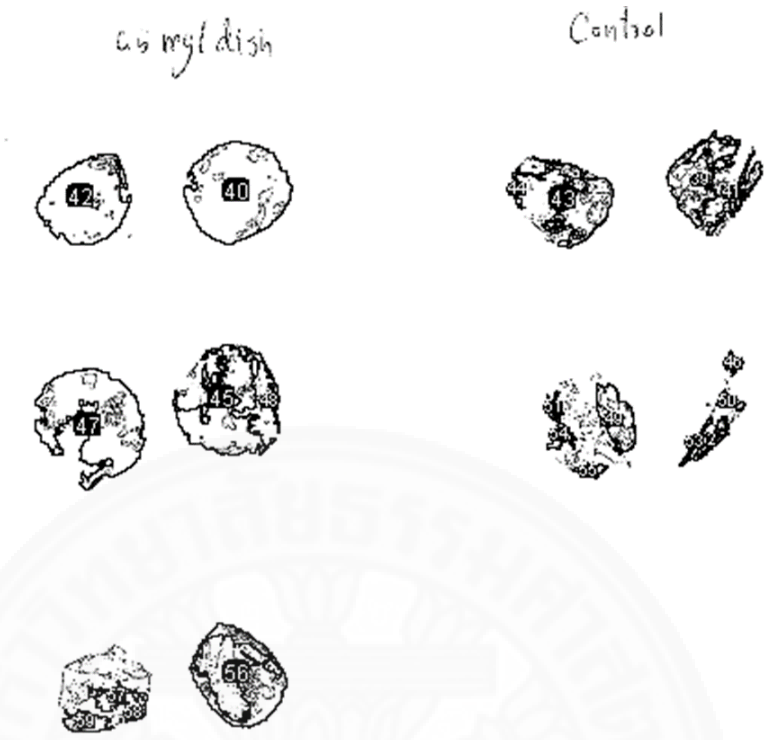


Figure B.11 The remaining areas of sweet potato leaves are coated with compound **64** after being eaten by the worms.

APPENDICE C

^{1}H-NMR SPECTROSCOPY

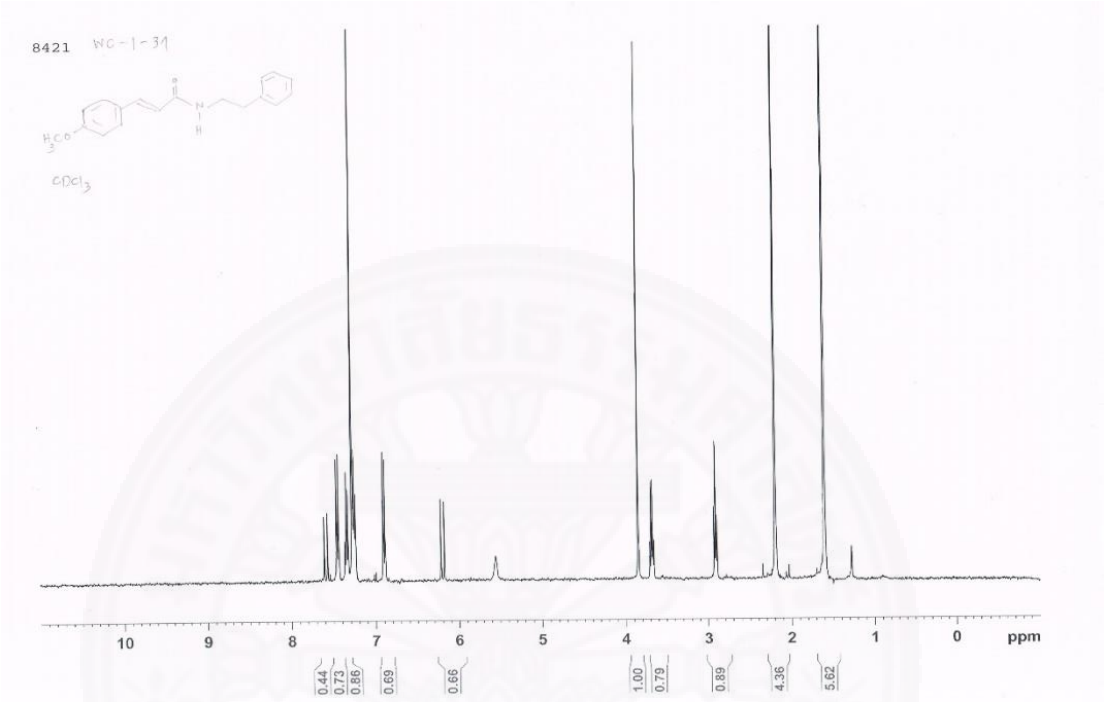


Figure C.1 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **41b.**

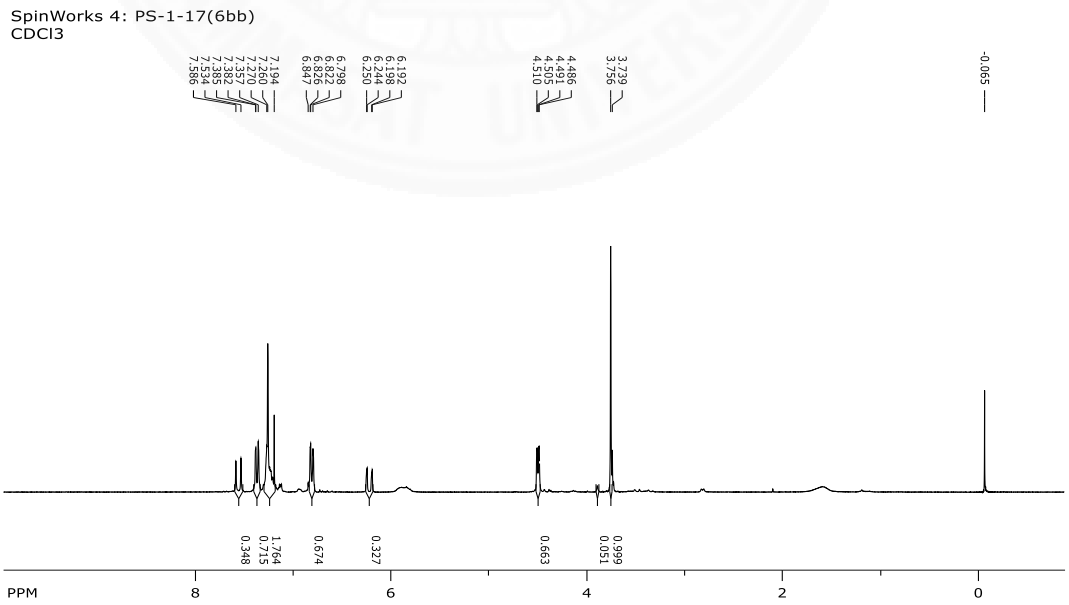


Figure C.2 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **49b.**

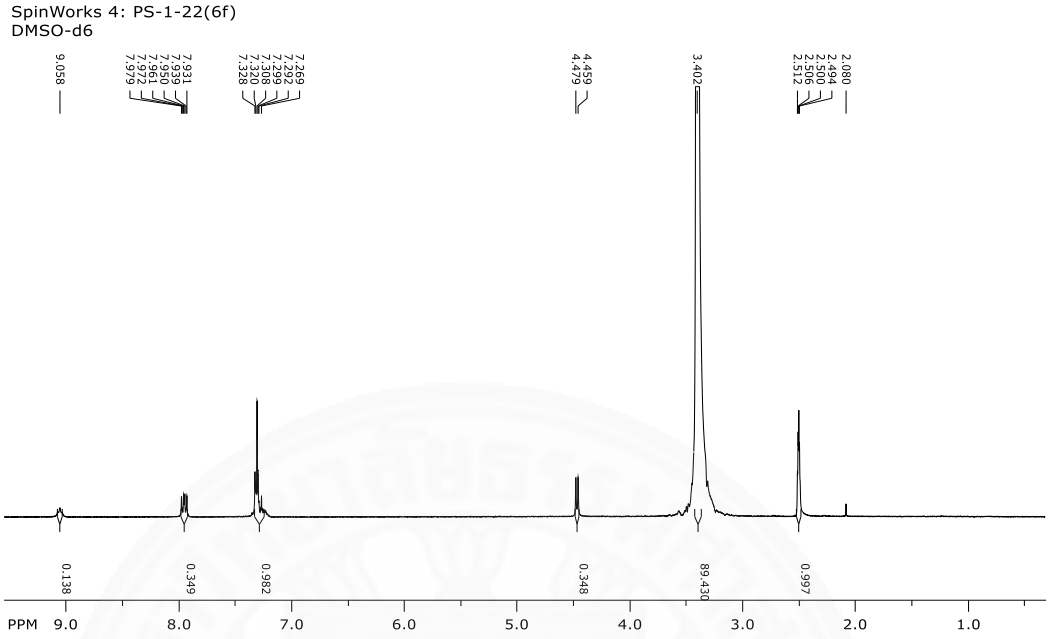


Figure C.3 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **50.**

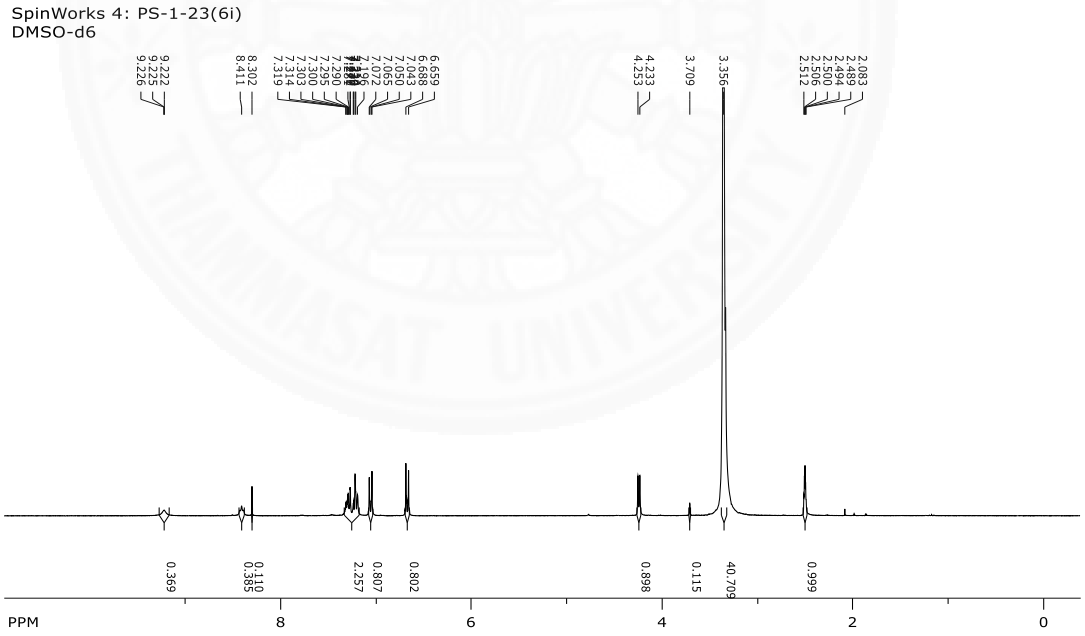


Figure C.4 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **51.**

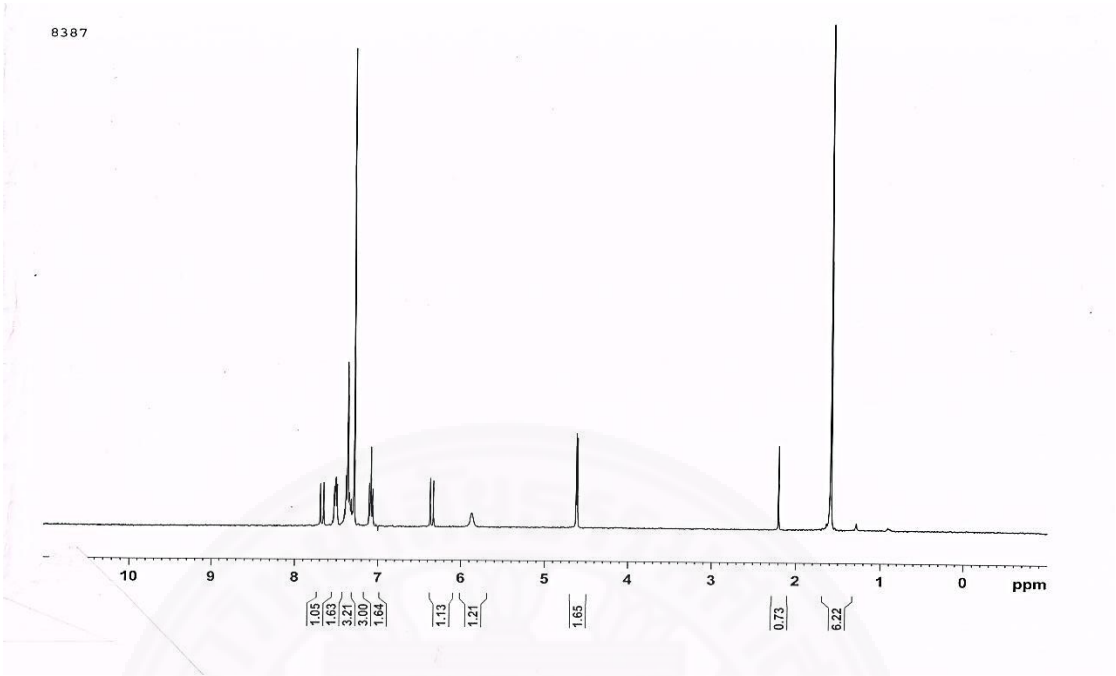


Figure C.5 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **52.**

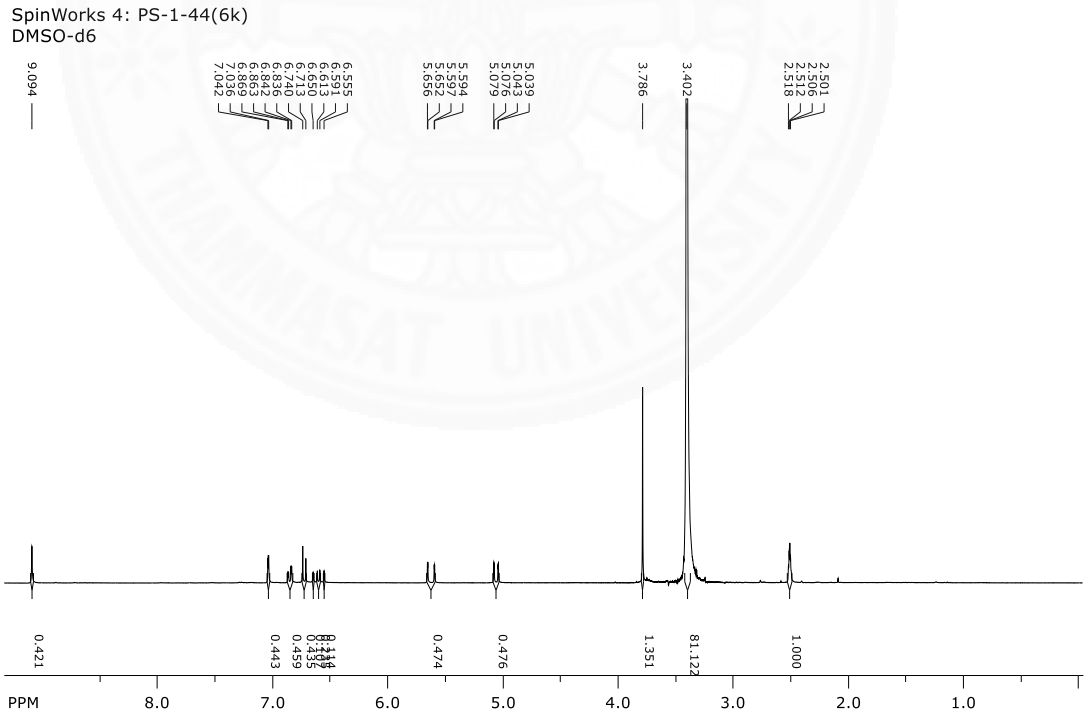


Figure C.6 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **53.**

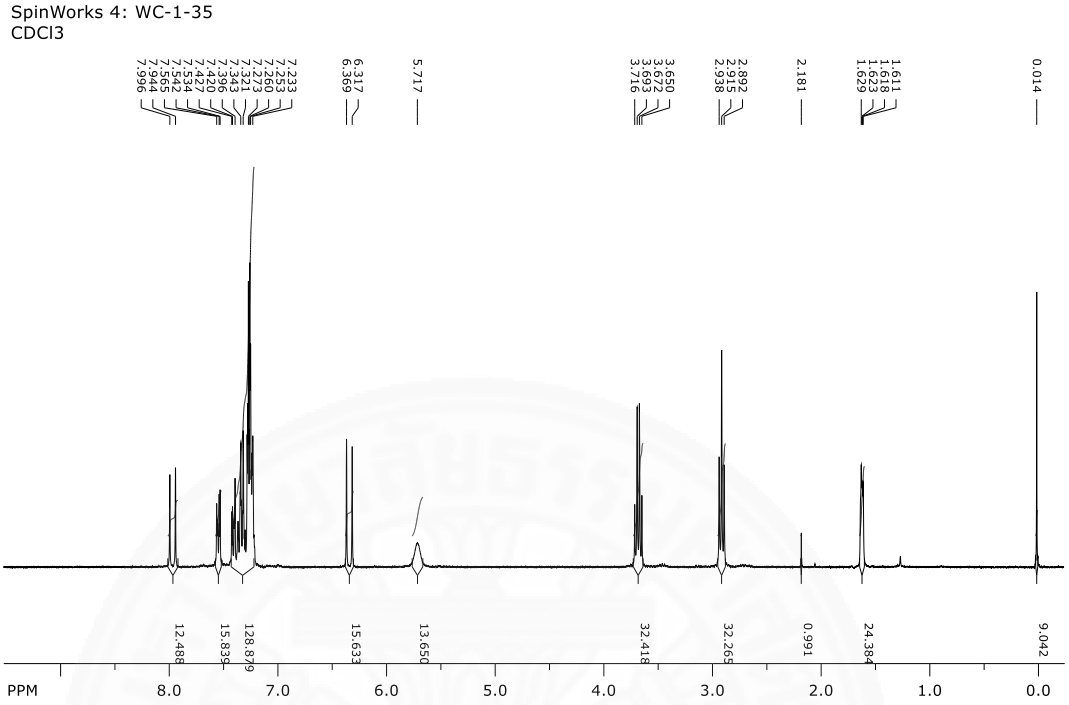


Figure C.7 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **54.**

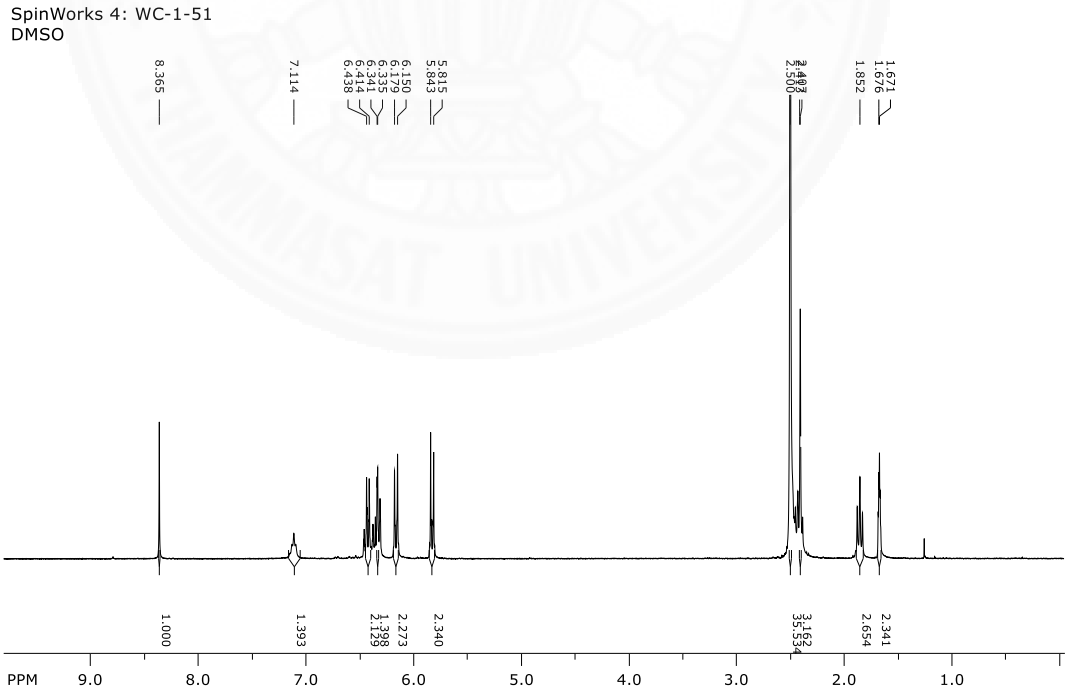


Figure C.8 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **55.**

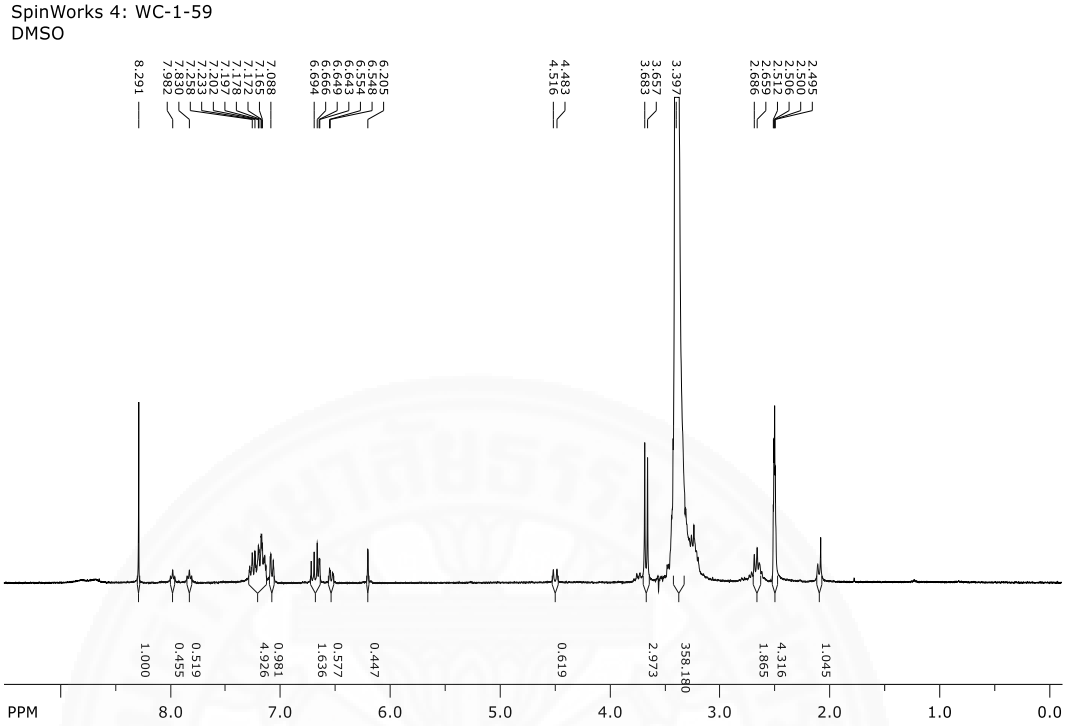


Figure C.9 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **56.**

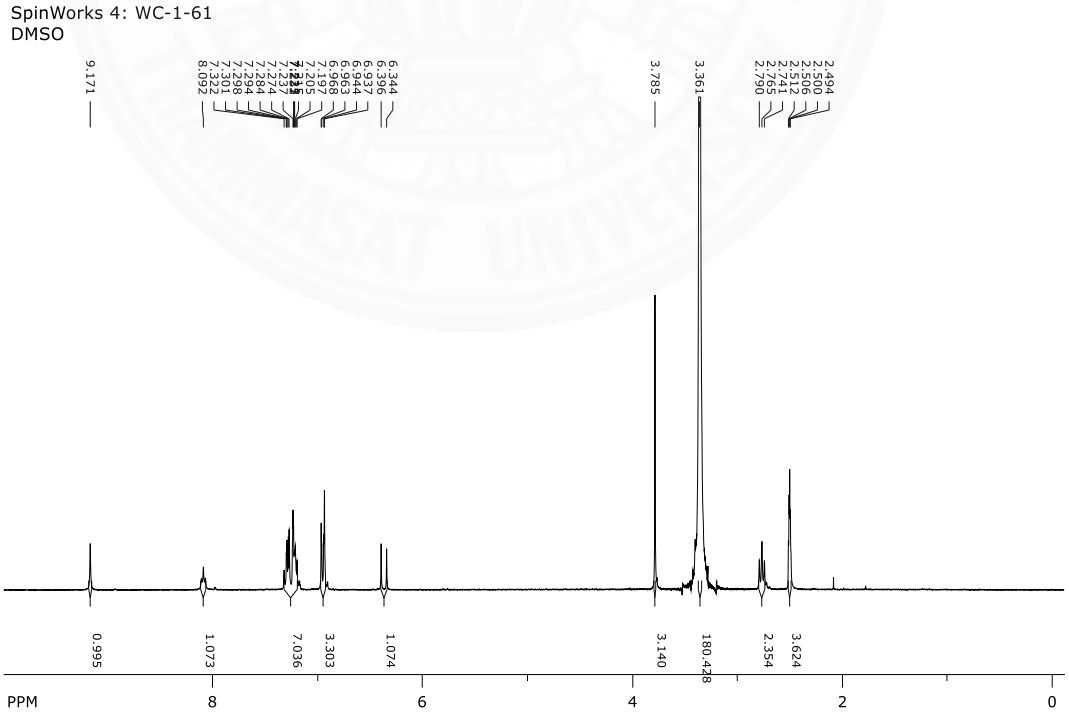


Figure C.10 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **57.**

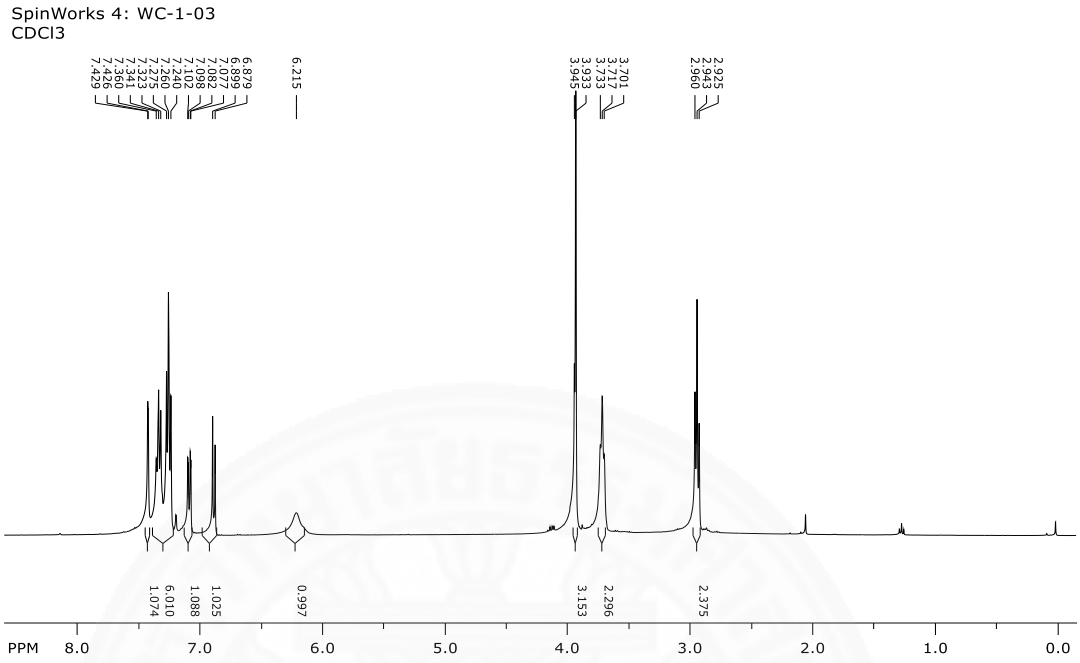


Figure C.11 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **58.**

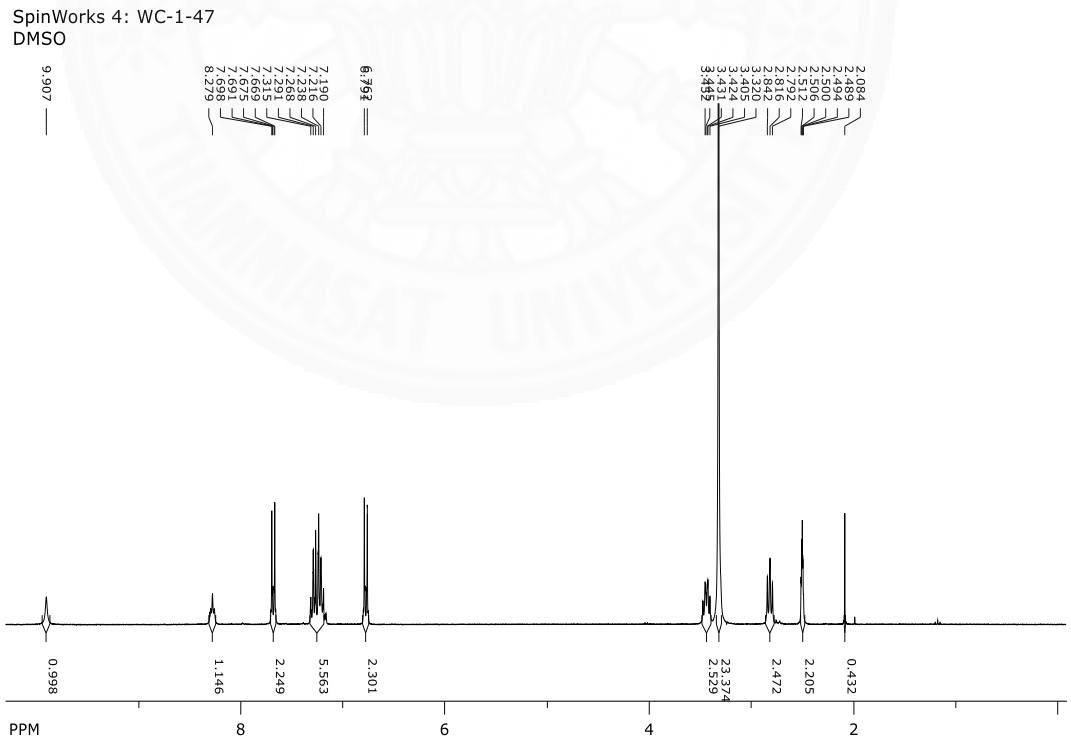


Figure C.12 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **59.**

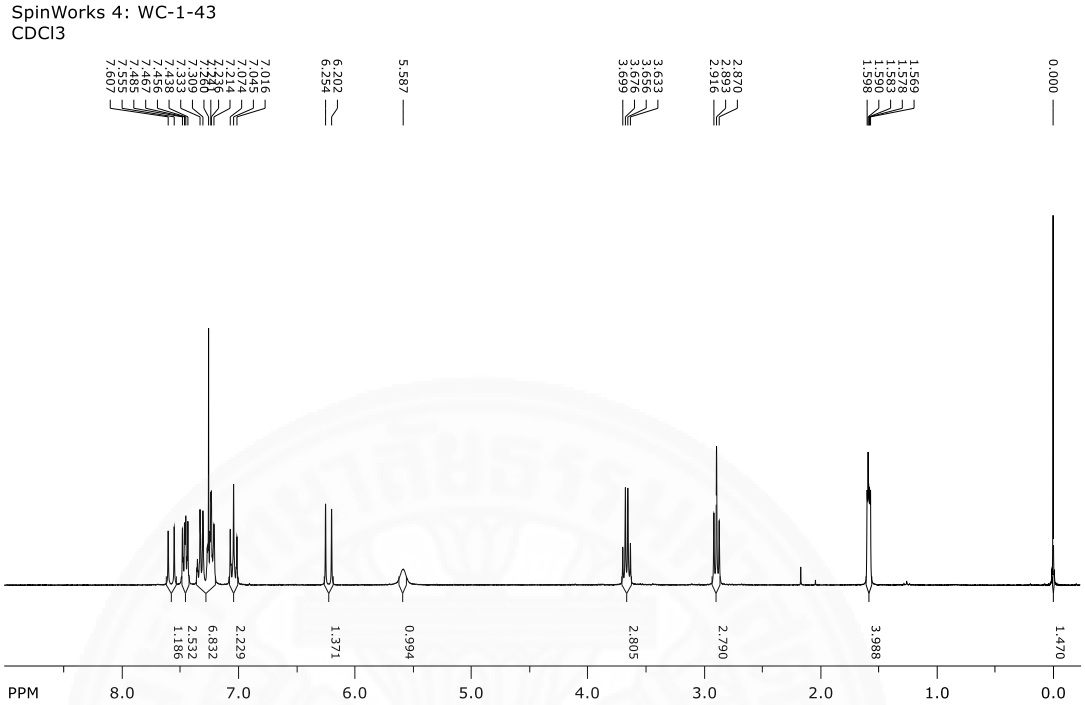


Figure C.13 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **60.**

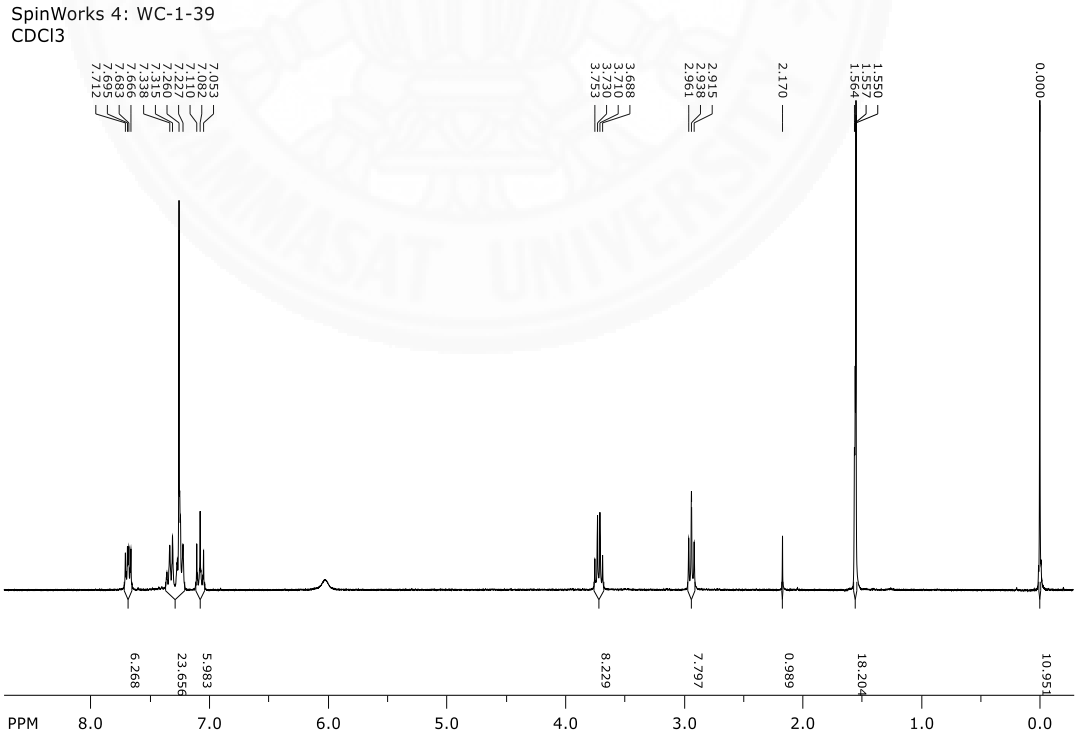


Figure C.14 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **61.**

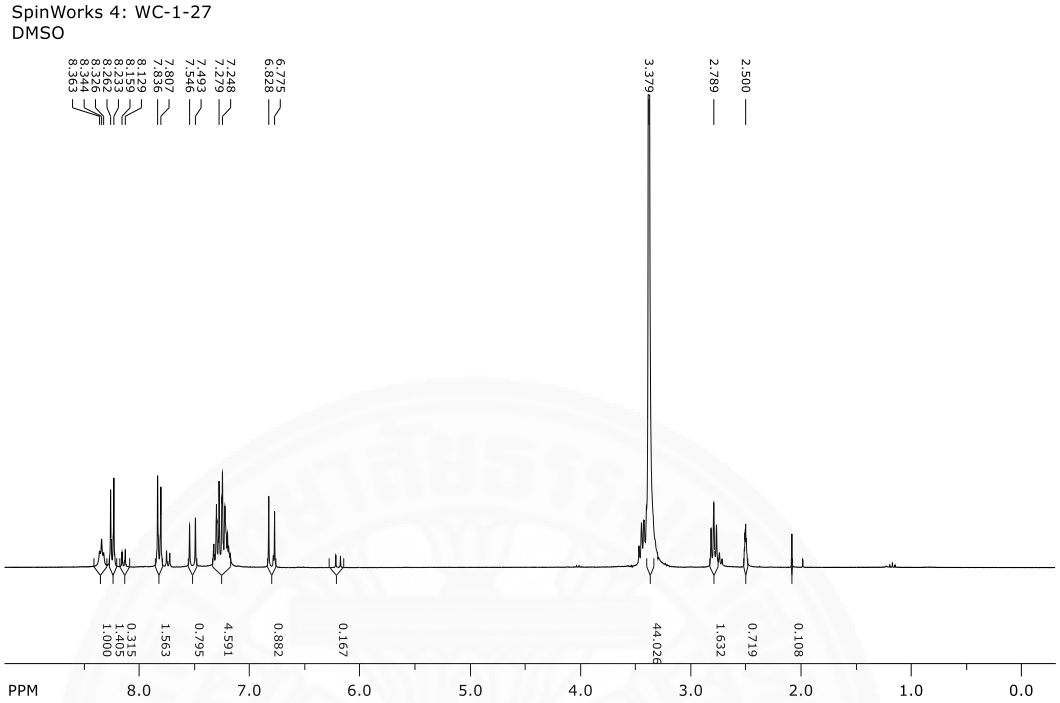


Figure C.15 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **62.**

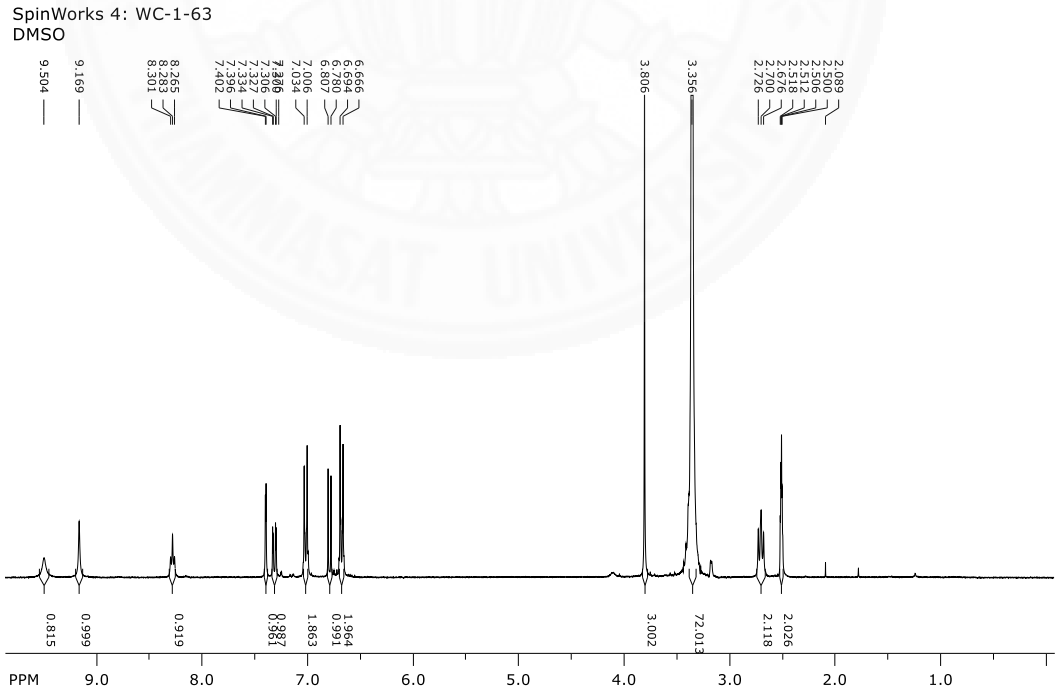


Figure C.16 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **63.**

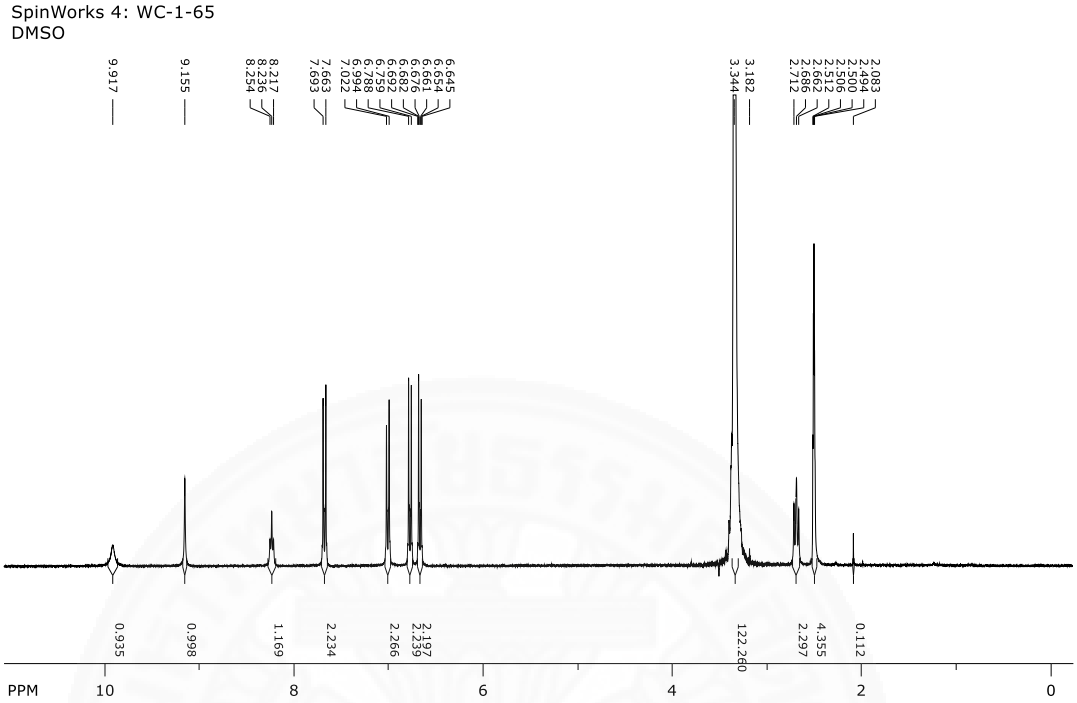


Figure C.17 ^{1}H-NMR (400 MHz, $CDCl_3$) of compound **64.**

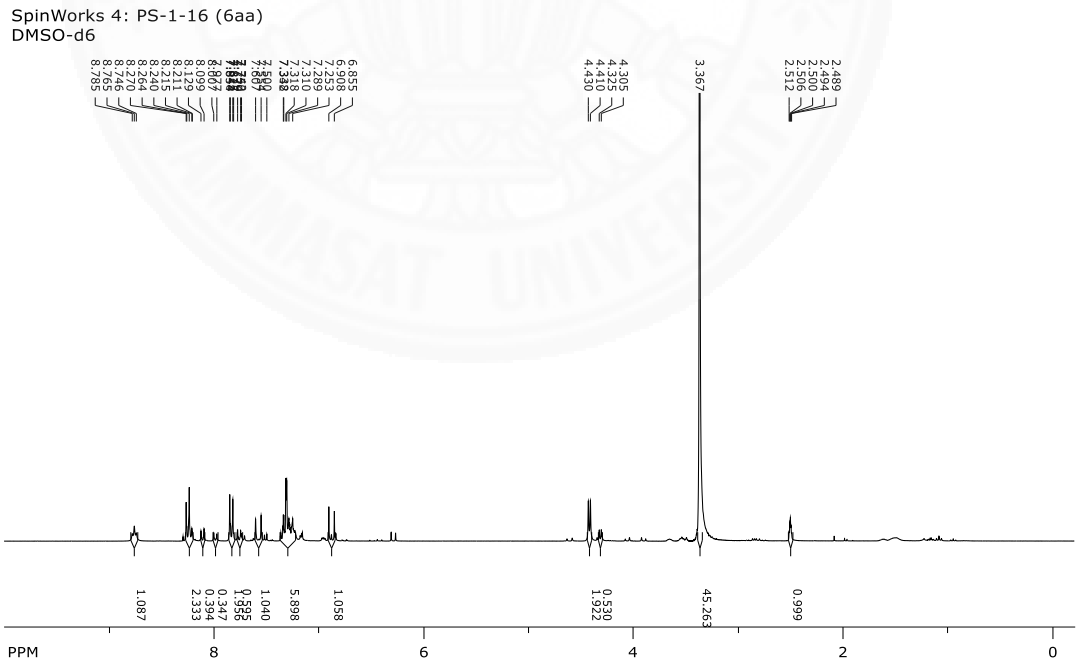


Figure C.18 ^{1}H-NMR (400 MHz, DMSO-d_6) of compound **65.**

BIOGRAPHY

Name	Miss Pailin Peththai
Date of Birth	August 23, 1993
Educational Attainment	2015: B.Sc. (Chemistry) Department of Science and Technology, Faculty of Chemistry, Thammasat University
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