SYNTHESIS OF COUMARINS AS CARBONIC ANHYDRASE II AND α-GLUCOSIDASE INHIBITORS

Miss Truc Phan Thi Hong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2019

การสังเคราะห์สารกลุ่มคูมารินเพื่อเป็นสารยับยั้งคาร์บอนิกแอนไฮเดรส II และแอลฟา-กลูโคซิเดส

น.ส.ตรุก พัน ทิ ฮง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	SYNTHESIS OF COUMARINS AS CARBONIC ANHYDRASE II AND α-GLUCOSIDASE INHIBITORS
By	Miss Truc Phan Thi Hong
Field of Study	Chemistry
Thesis Advisor	Assistant Professor WARINTHORN CHAVASIRI, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the Faculty of Science
(Professor POLKIT SANGVANICH, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor VUDHICHAI PARASUK, Ph.D.)

..... Thesis Advisor
(Assistant Professor WARINTHORN CHAVASIRI, Ph.D.)

..... Examiner
(Assistant Professor ANAWAT AJAVAKOM, Ph.D.)

..... External Examiner
(Assistant Professor Veena Satitpatipan, Ph.D.)

ตรุก พัน ทิ ฮง : การสังเคราะห์สารกลุ่มคูมารินเพื่อเป็นสารยับยั้งคาร์บอนิกแอนไฮเดรส II และแอลฟา-กลูโคซิเดส. (SYNTHESIS OF COUMARINS AS CARBONIC ANHYDRASE II AND α-GLUCOSIDASE INHIBITORS) อ.ที่ปรึกษาหลัก : ผศ. ดร.วรินทร ชวศิริ

คูมารินมีความสำคัญในเคมีทางยา ในงานวิจัยนี้ ได้ทดสอบฤทธิ์ของอนุพันธ์อัมเบลลิเฟอโรนต่อการยับยั้งเอนไซม์สองชนิด ได้แก่ คาร์บอนิกแอนไฮเดรส (CA II) และแอลฟา-กลูโคซิเดส การศึกษาส่วนแรก ได้สังเคราะห์ พิสูจน์ทราบโครงสร้างและทดสอบฤทธิ์ยับยั้ง CA II ของอนุพันธ์อีเทอร์ โบรมิเนเทตอีเทอร์และเอสเทอร์ อย่างไรก็ตามผลการทดสอบไม่สามารถได้ข้อสรุปความสัมพันธ์ของโครงสร้างและฤทธิ์ทางชีวภาพ เนื่องจากผลการทดสอบไม่สามารถทำซ้ำได้ ในส่วนที่สอง ได้สังเคราะห์และศึกษาฤทธิ์ยับยั้งแอลฟากลูโคซิเดสของอนุพันธ์ของอัมเบลลิเฟอโรนสามสิบสี่ตัว (อนุพันธ์อีเทอร์ โบรมิเนเทตอีเทอร์ เอสเทอร์ ซัลโฟนาไมด์ และซัลโฟเนต) ได้ค้นพบว่า ส่วนที่ไม่ชอบน้ำส่งผลต่อฤทธิ์ยับยั้งแอลฟา-กลูโคซิเดส นอกจากนี้อนุพันธ์อีเทอร์ของอัมเบลลิเฟอโรนที่มีหมู่โบรมีนแทนที่ที่ตำแหน่งที่ 3 และหมู่แทนที่ซัลโฟนาไมด์แสดงฤทธิ์ยับยั้งแอลฟา-กลูโคซิเดสที่ดีขึ้น สารใหม่ 45 65 และ 70 แสดงฤทธิ์ยับยั้งที่ดีด้วยค่า IC_{50} 68, 88 และ 37 μM ตามลำดับ ซึ่งดีกว่าสารควบคุม acarbose (IC_{50} 94 μM).

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2562 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6072201323 : MAJOR CHEMISTRY
KEYWORD: coumarins; umbelliferone; carbonic anhydrase II; alpha-glucosidase

Truc Phan Thi Hong : SYNTHESIS OF COUMARINS AS CARBONIC ANHYDRASE II AND α-GLUCOSIDASE INHIBITORS. Advisor: Asst. Prof. WARINTHORN CHAVASIRI, Ph.D.

Coumarins have been occupied an important position in medicinal chemistry. In this research, umbelliferone derivatives were evaluated for inhibitory activity of two enzymes, namely carbonic anhydrase II (CA II) and α-glucosidase. First, ether, brominated ether and ester derivatives of umbelliferone were synthesized, characterized and tested for CA II inhibitory activity. However, the inhibition data could not be used to make the exact conclusion about structure-activity relationship owing to their unrepeatable results. For the second part, thirty-four derivatives of umbelliferone (ether, brominated ether, ester, sulfonamide and sulfonate derivatives) were synthesized and explored for anti-α-glucosidase activity. It was disclosed that anti-α-glucosidase activity was affected by hydrophobic effect. In addition, bromo substituent at position 3 of ether derivatives of umbelliferone and sulfonamide substituents could improve anti-α-glucosidase activity. Three new compounds 45, 65 and 70 demonstrated good inhibitory activities with IC_{50} of 68, 88, and 37 μM, respectively, which were better than that of a positive control, acarbose (IC_{50} 94 μM).

Field of Study: Chemistry Student's Signature .....
Academic Year: 2019 Advisor's Signature .....

ACKNOWLEDGEMENTS

The author would like to express sincere gratitude to her advisor Assistant Professor Dr. Warinthorn Chavasiri for the continuous support of her master study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped her in all the time of research and writing of this thesis.

Besides her advisor, she would like to thank the rest of the thesis committee: Associate Professor Dr. Vudhichai Parasuk, Assistant Professor Dr. Anawat Ajavakom, and Assistant Professor Dr. Veena Satitpatipan for their encouragement, insightful comments, suggestions to complete her thesis.

The author wishes to thank for guidance and encouragement of Dr. Duong Thuc Huy in her study.

Appreciations were given to Asean Scholarship Chulalongkorn University 2016 and 90th Anniversary of Chulalongkorn University Fund which supported her financially, chemical and laboratory equipment throughout two-year study in Thailand.

Moreover, the author wants to give a sincerely thankfulness to PhD students Kieu Van Nguyen, Trang Hoang Thuy Thuy Le, Thao Thanh Nguyen Huynh, Asshaima Paramita Devi, Master students Duy Vu Nguyen, Dung Thi Kim Le and other Vietnamese friends as well as Thai, Indonesia and Japanese friends for their supporting and inspiration.

Specially, the author wants to express her grateful to her parents as well as her family in Vietnam for giving birth to her, encouraging and motivating in her life.

Truc Phan Thi Hong

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABREVIATION

δ	chemical shift
δ_C	chemical shift of carbon
μg	microgram(s)
δ_H	chemical shift of proton
μM	micromolar
$[M+K]^+$	pseudomolecular ion
$[M+Na]^+$	pseudomolecular ion
AGI(s)	α-Glucosidase inhibitor(s)
AZA	Acetazolamide
br	Broad signal
CA(s)	Carbonic anhydrase(s)
CAI(s)	Carbonic anhydrase inhibitor(s)
d	doublet (NMR)
dd	doublet of doublets (NMR)
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
ED	Effective dose
eq	Equivalent
ESI	Electron spray ionization
EtOAc	Ethyl acetate
g	Gram(s)
h	hour(s)
HRMS	High resolution mass spectra
IC	Inhibition concentration
IDF	International Diabetes Federation
m	Multiplet (NMR)
MeCN	Acetonenitrile
MHz	Mega Hertz

mL	Milliliter(s)
mmol	Millimole(s)
NA	No activity
p-NPG	*p*-nitrophenyl-**α**-*D*-glucosidase
q	Quartet (NMR)
quint	Quintet (NMR)
rt	Room temperature
s	Singlet (NMR)
sext	Sextet (NMR)
t	Triplet (NMR)
TLC	Thin layer chromatography
WHO	World Health Organization
α	Alpha

CHAPTER 1

COUMARINS AND THEIR BIOACTIVITIES

Coumarins (or benzopyran-2-one), a very large series of polyphenolic derivatives, can be classified into different categories such as simple coumarin prototype and many other groups of polycyclic analogs (furanocoumarins and pyranocoumarins).[1] The most widely diffuse derivatives from natural coumarins are umbelliferone, esculetin and scopoletin (**Figure 1.1**).[1] In addition, coumarins play an important role in medicinal chemistry ascribed to their ability to exert noncovalent interaction (π-π, hydrophobic, electrostatic interactions, hydrogen bonds, metal coordination and van der Waals force *etc.*) with various active sites in organisms.[2] Recently, numerous studies have been proven about multiple potential activities of coumarins including anti-proliferative[3], anti-cancer[4, 5], anti-HCV[6], anti-HIV[7], anti-Alzheimer[8], anti-malarial[9, 10], anti-bacterial[11], anti-fungal[12], anti-oxidant[13], anticonvulsant[14], anti-inflammatory[15], enzyme inhibition[16] and so on. Moreover, coumarin based derivatives used for therapeutic purposes in clinic are listed below such as acenocoumarol, dicoumarolum, warfarin and carbochromen (**Figure 1.1**).[17] Coumarin has achieved a central position as an advantage structure in the design and discovery of novel drug molecules because it has high affinity and specificity to different molecular targets.[18] Coumarin contains a planar aromatic ring fused with lactone functionality, a readily available group for hydrogen bonding as well as for protein-ligand interaction.[19] These key features make this heterocycle a unique pharmacophore in medicinal

chemistry arena.[19] Furthermore, hybrid multifunctional molecules may have better inhibitory activities, improved selectivity profile, different or dual modes of action and/or reduced undesired side effects.[16] Many studies have designed and accessed coumarin-hybrid compounds which display different pharmacological properties.[16] These molecules incorporate coumarin and other heterocyclic and non-heterocyclic scaffolds. These compounds were reported to inhibit several enzymes including cholinesterase, monoamine oxidase (A & B), glucosidase, aldehyde/aldose reductase, alkaline phosphatase, urease, carbonic anhydrase, lysine specific demethylase, histone deacetylase, lipoxygenase, topoisomerase, tyrosinase and cyclooxygenase.[16] The inhibitory activities of the synthesized coumarin compounds towards the carbonic anhydrase II (CA II) and **α**-glucosidase were investigated and reported here in.

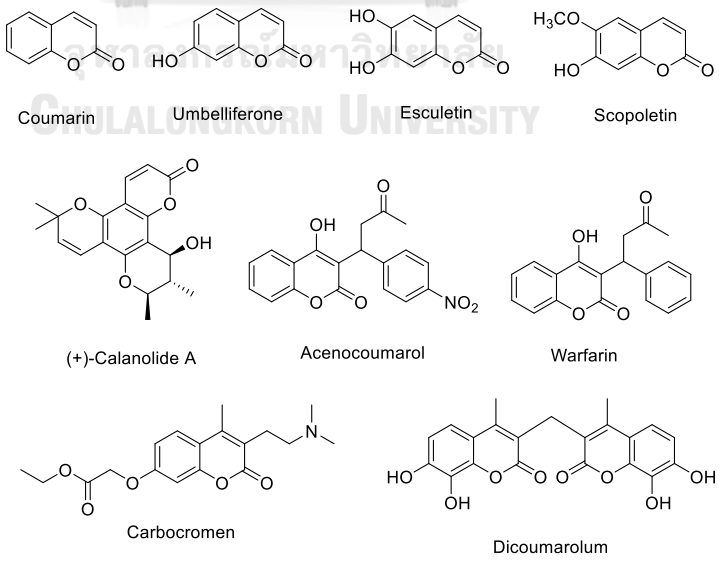


Figure 1.1 Structures of coumarin, (+)-Calanolide A and drugs based on coumarin moiety.

CHAPTER 2

SYNTHESIS OF COUMARINS AS CARBONIC ANHYDRASE II INHIBITORS

2.1 Introduction

2.1.1 Carbonic anhydrase and carbonic anhydrase II

Carbonic anhydrases (CAs) are a family of zinc metalloenzymes, which catalyze the interconversion between CO_2 and HCO_3^-. As such, CAs is important for several physiological processes such as pH regulation, CO_2 homeostasis, respiration, bone resorption, and tumorigenesis. Sixteen different **α**-CA isoforms were isolated in mammals, which are cytosolic (CA I, CA II, CA III, CA VII, CA XIII), membrane-bound (CA IV, CA IX, CA XII, CA XIV and CA XV), CA Va and CA Vb mitochondrial, and CA VI secreted in saliva and colostrum. In addition, there are three catalytically inactive forms (CA VIII, CA X, and CA XI) referred to as CA-related proteins (CARPs).[20-22] The CA II is involved in several diseases, such as glaucoma, edema, epilepsy, and probably altitude sickness.[23]

The **α**-CA active site structure is conserved and is conically shaped with a zinc atom located at the base, which is coordinated by three histidine residues (His94, His96, His119) and a water/hydroxide ion. The catalytic mechanism of CA occurs in two steps. In the hydration direction, first there is a nucleophilic attack of CO_2 by the zinc-bound hydroxide ion, resulting in a zinc-bound bicarbonate that is subsequently displaced by H_2O.[21, 24, 25] The enzyme active site is then regenerated to a zinc-bound hydroxide ion by a proton transfer mechanism from the zinc-bound water molecule

to bulk solvent, facilitated by an ordered water wire within the active site and His64 acting as a proton donor/acceptor shuttle residue.[26-29]

2.1.2 Glaucoma disease

Glaucoma is an optic neuropathy defined by characteristic optic disc damage and visual field loss for which increased intraocular pressure (IOP) is a major modifiable risk factor.[30] Moreover, glaucoma is the second leading cause of blindness in worldwide and is the world's most common cause of irreversible blindness.[30, 31] According to Glaucoma Research Foundation, approximately 10% of people with glaucoma who receive proper treatment still experience loss of vision. Everyone is at risk for glaucoma from babies to senior citizens. Older people are at a higher risk, but babies can be born with glaucoma (approximately 1 out of every 10,000 babies born in the United States). Young adults can get glaucoma, too.

Glaucoma can be categorized into 2 major subtypes, open angle and angle closure, both of which result in characteristic optic nerve degeneration. Both can be further subdivided into primary or secondary due to some other inciting factors. Secondary glaucoma can result from many other pathologic processes, including but not limited to vasculopathic, malignant, and traumatic.[30]

Open-angle glaucoma is a chronic, insidious process which is also called primary or chronic glaucoma. "Open-angle" means that the angle where the iris meets the cornea is as wide and open as it should be. Open-angle glaucoma is caused by the

slow clogging of the drainage canals, resulting in increased eye pressure. Patients are often unaware of their disease until vision loss has progressed significantly, known as the “sneak thief of sight.” Early diagnosis remains a challenge given the insidious nature of the onset of this process and, therefore, formal ophthalmologic evaluation of any patient with risk factors is critical for prompt detection.[30] Normal and glaucoma anatomies as well as open-angle glaucoma were illustrated in **Figure 2.1.**

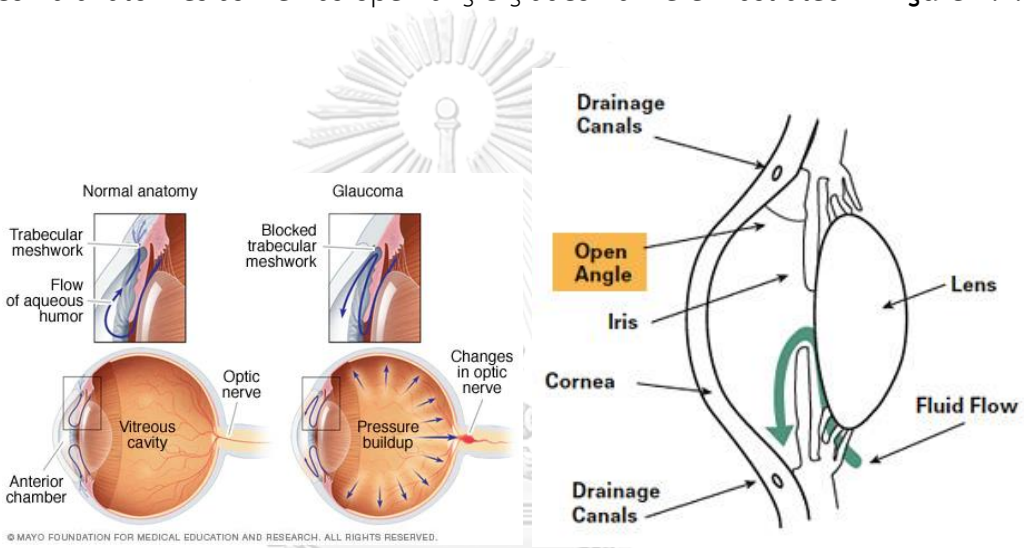


Figure 2.1 Open-angle glaucoma (www.mayoclinic.org, www.glaucoma.org)

In contrast, angle-closure glaucoma can be an acute process with more immediate signs and symptoms but may also present insidiously and tends to be a more visually destructive subtype. It is also called acute glaucoma or narrow-angle glaucoma. The angle-closure glaucoma accounts for approximately half the cases of glaucoma worldwide and, when acute, is considered an ocular emergency because loss of vision can occur within hours to days. By 2020, it is estimated that there will be 21 million cases of primary angle-closure glaucoma, with 5.3 million blinds

bilaterally. Unlike the open-angle glaucoma, the angle-closure glaucoma is a result of the angle between the iris and cornea closing.[30, 32] Angle-closure glaucoma anatomy were introduced in **Figure 2.2**.

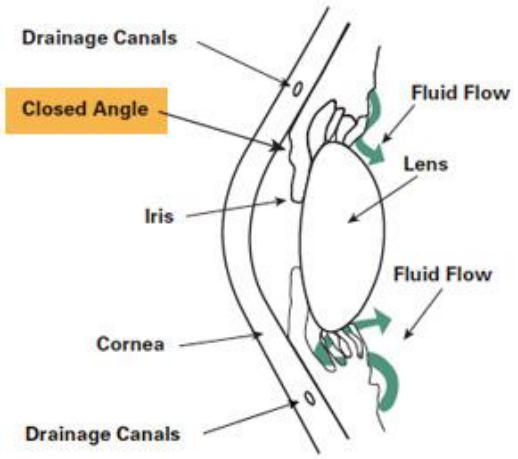


Figure 2.2 Closed angle glaucoma (www.glaucoma.org)

To treat glaucoma, there are many options including eye drops, laser procedures, and surgery.[33] All are intended to decrease eye pressure and, thereby, protect the optic nerve.[34] Carbonic anhydrases catalyze the reversible hydration of CO_2 and the dehydration of carbonic acid. In the eye, it lowers IOP by decreasing the formation of aqueous humor.[35] Therefore, carbonic anhydrase II inhibitors can reduce formation of bicarbonate ions which reduce IOP.

2.1.3 Inhibition of carbonic anhydrase II of sulfonamides

CA inhibitors are sulfonamides, their bioisosteres (sulfamates, sulfamides, *etc.*), metal complexing anions, phenols, and thiophenols.[20, 21, 36] Sulfonamides as classical carbonic anhydrase inhibitors have been used as commercial drugs for treatment of

glaucoma.[21] However, they also inhibit all CA isoforms nonspecifically, diluting the drug effectiveness and causing undesired side effects from the off-target inhibition.[21] Therefore, further examination on this enzyme continues capturing the attentions of drug discovery scientists.

2.1.4 Inhibition of carbonic anhydrase II of coumarins

Recently, coumarins were a novel class of inhibitors of the CA because they are considered prodrugs that can only bind the enzyme in the form of their hydrolysis products (**Figure 2.3**).[37, 38] Coumarins are proposed to first bind in the active site undergo hydrolysis due to the esterase activity of CA, and then reorient to prevent steric hindrance. Due to this required chemical transformation, coumarins are considered suicide compounds and their inhibitory properties are time-dependent.[38] The hydrolyzed form of the inhibitor binds at the entrance of the active site, blocking substrate entry and preventing catalysis.[37, 38] Since coumarins bind near or within the selective pocket where majority of unique residues are located, this class provides a strong scaffold for the design of isoform selective inhibitors. It has been demonstrated that isoform specificity improves with the addition of chemical substituents to the coumarin scaffold, with the significance of improvement dependent on the number and chemical nature of the functional groups.[37]

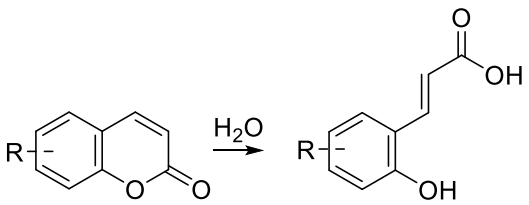
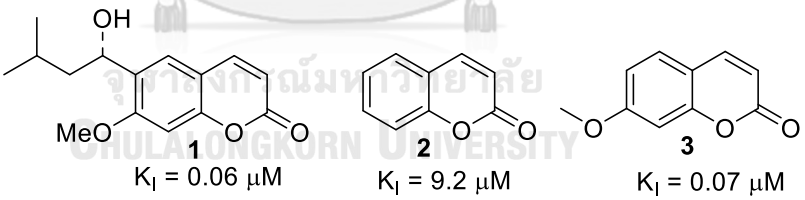
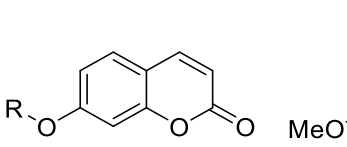


Figure 2.3 Coumarins and hydrolysis product

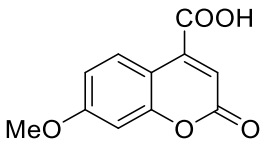
Various substituted coumarins were studied for their anticarbonic anhydrase II activity. In 2009, Maresca *et al*. investigated the inhibition profile against the mammalian isozyme CA II with coumarins **1-3**. **1** is a natural product isolated from Australian plant *Leionema ellipticum* Paul G. Wilson (Rutaceae) and revealed the most effective inhibitor of CA II with an inhibition constant K_I = 0.06 μM. Based on the difference between number of substituents on the aromatic coumarin scaffold of three compounds, the researchers suggested that substitutions lead to increase in the CA II inhibition activity.[37]



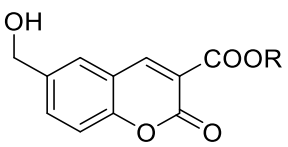
In 2010, Maresca *et al*. studied a series of substituted coumarins (**4**-**12**). The best CA II inhibitors were methyl ester **11** and carboxylic acid **9**. Monosubstituted derivatives **3-8** incorporating bulky chains at position 7 decreased the activity. Among **10**-**12**, there is an enormous difference of activity which indicated important role of CH_2 group in structure-activity relationship.[38]



4: R = Et; K_I = 12.4 μM
5: R = n-Pr; K_I = 145 μM
6: R = n-Bu; K_I = 213 μM
7: R = $PhCH_2$; K_I = 224 μM
8: R = $PhCH_2CH_2$; K_I = 243 μM

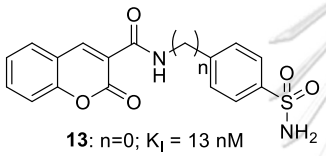


9: K_I = 0.099 μM

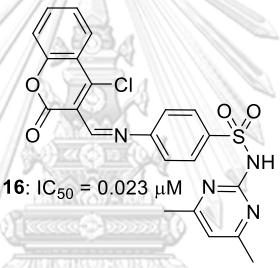


10: R = H; K_I = 44.5 μM
11: R = Me; K_I = 0.032 μM
12: R = Et; K_I = 50.5 μM

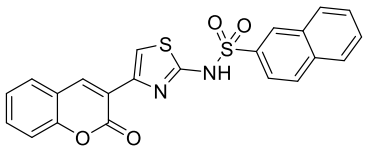
In addition, sulfonamide coumarins have been investigated and disclosed the inhibition effect against CA II. These findings encouraged researchers to explore the synthesis of sulfonamide coumarins and to evaluate their activities.[39-41]



13: n=0; K_I = 13 nM
14: n=1; K_I = 12 nM
15: n=2; K_I = 10 nM

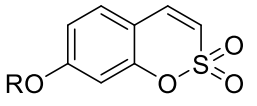


16: IC_{50} = 0.023 μM

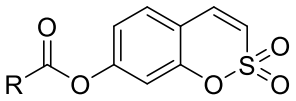


17: IC_{50} = 8.48 μM

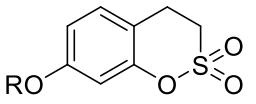
Sulfocoumarins have been discovered to reveal significant inhibition towards CA II. In 2013, sulfocoumarins (**18**-**33**) with a substituent at position 7 were synthesized and displayed specificity for CA II with low nanomolar binding affinities, which is better than acetazolamide - an antiglaucoma drug (K_I = 12 nM). These results supported strong potential of 7-substituted sulfocoumarins as a novel glaucoma therapy.[42]



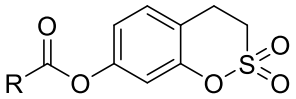
18: R = H; K_I = 8.4 nM
19: R = Me; K_I = 1.5 nM
20: R = Bn; K_I = 2.5 nM
21: R = Ms; K_I = 2.1 nM



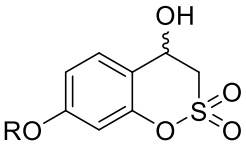
22: R = Me; K_I = 2.2 nM
23: R = 4-chlorobenzyl; K_I = 2.6 nM
24: R = 2-bromobenzyl; K_I = 1.9 nM



25: R = H; K_I = 7.6 nM
26: R = Me; K_I = 2.4 nM
27: R = Bn; K_I = 2.0 nM
28: R = Ms; K_I = 1.8 nM



29: R = Me; K_I = 1.5 nM
30: R = 4-chlorobenzyl; K_I = 2.2 nM
31: R = 2-bromobenzyl; K_I = 3.4 nM



32: R = H; K_I = 1.6 nM
33: R = Bn; K_I = 2.3 nM

2.1.5 The aim of this study

In previous works, researchers investigated the effect of substituents on coumarin scaffold to CA II inhibitory activity such as number of substituents, bulky chains at position 7, carboxyl group at position 3. In addition, many sulfonamide coumarins and sulfocoumarins as CA II inhibitors were investigated. All these findings encouraged us to explore the synthesis of various substituted coumarins such as ether, brominated ether, ester, sulfonamide coumarins. These derivatives would further be evaluated for CA II inhibitory activity as well as analyzed for structure-activity relationship.

2.2 Experimential section

2.2.1 General procedure

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, acetone-d_6 or DMSO-d_6 using a Bruker Ultrashield 400 Plus NMR spectrometer or a Varian Mercury NMR spectrometer with an Oxford YH400 magnet operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. High

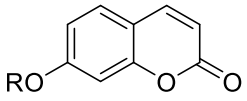
resolution mass spectra (HRMS) were recorded on a Bruker Daltonics microTOF using electron spray ionization (ESI). All solvents used in this research were distilled prior to use except those which were reagent grades. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 PF254). Merck silica gel (No. 7734) was used as stationary phase on open column chromatography.

2.2.2 Synthesis of coumarins

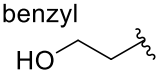
A Ether and brominated ether derivatives of umbelliferone

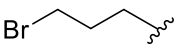
A1 Ether derivatives

A mixture of umbelliferone (**34**, 5 mmol), alkyl halide (2 eq), and K_2CO_3 (3 eq) in DMF (10 mL) was stirred at 55–60 ^{0}C for times ranging from 5 h to overnight. The reaction mixture was extracted with EtOAc, and the combined extracts were washed with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue then was purified by column chromatography on silica gel with *n*-hexane/EtOAc to yield **3**, **4**, **7** and **35-41**.[43]



34: R = H
3 : R = CH_3
4 : R = C_2H_5
35: R = C_4H_9
36: R = C_8H_{17}
37: R = $C_{12}H_{25}$

38: R = allyl
39: R = prenyl
7 : R = benzyl
40: R = 

41: R = 

7-methoxy-2*H*-chromen-2-one (**3**, 93 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.86 (s, 3H), 6.23 (d, 1H, *J* = 9.2 Hz), 6.79 (d, 1H, *J* = 2.4 Hz), 6.83 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.36 (d, 1H, *J* = 8.8 Hz), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 55.9, 101.0, 112.7 (2C), 113.2, 128.9, 143.5, 156.0, 161.3, 163.0.

7-ethoxy-2*H*-chromen-2-one (**4**, 84 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.44 (t, 3H, *J* = 7.2 Hz), 4.07 (q, 2H, *J* = 7.2 Hz), 6.22 (d, 1H, *J* = 9.6 Hz), 6.78 (d, 1H, *J* = 2.4 Hz), 6.81 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.34 (d, 1H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 9.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.7, 64.5, 101.4, 112.5, 113.0 (2C), 128.8, 143.5, 156.0, 161.3, 162.4.

7-butoxy-2*H*-chromen-2-one (**35**, 91% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.98 (t, 3H, *J* = 7.6 Hz), 1.49 (sextet, 2H, *J* = 7.6 Hz), 1.79 (quint, 2H, *J* = 6.4 Hz), 4.00 (t, 2H, *J* = 6.8 Hz), 6.22 (d, 1H, *J* = 9.6 Hz), 6.79 (s, 1H), 6.82 (d, 1H, *J* = 8.8 Hz), 7.34 (d, 1H, *J* = 8.8 Hz), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 13.9, 19.3, 31.3, 68.5, 101.5, 112.5, 113.0, 113.1, 128.8, 143.5, 156.1, 161.4, 162.6.

7-(octyloxy)-2*H*-chromen-2-one (**36**, 31 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* = 6.4 Hz), 1.31 (m, 8H), 1.46 (quint, 2H, *J* = 6.8 Hz), 1.80 (quint, 2H, *J* = 6.8 Hz), 4.00 (t, 2H, *J* = 6.8 Hz), 6.23 (d, 1H, *J* = 9.6 Hz), 6.79 (s, 1H), 6.82 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.35 (d, 1H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 9.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 26.1, 29.1, 29.3, 29.4, 31.9, 68.8, 101.5, 112.5, 113.0, 113.1, 128.8. 143.5, 156.1, 161.4, 162.6.

7-(dodecyloxy)-2*H*-chromen-2-one (**37**, 87 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* = 8.0 Hz), 1.26 (m, 16H), 1.46 (quint, 2H, *J* = 6.4 Hz), 1.80 (quint, 2H, *J* = 6.8 Hz), 4.00 (t, 2H, J = 6.4 Hz), 6.23 (d, 1H, *J* = 9.6 Hz), 6.79 (d, 1H, *J* = 2.0 Hz), 6.82 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.34 (d, 1H, *J* = 8.8 Hz), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 26.1, 19.1, 29.5, 29.7, 29.7, 29.8, 29.8, 32.0, 68.8, 101.5, 112.5, 113.1 (2C), 128.8, 143.5, 156.1, 161.4, 162.6.

7-(allyloxy)-2*H*-chromen-2-one (**38**, 23 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 4.58 (d, 2H, *J* = 5.2 Hz), 5.32 (d, 1H, *J* = 10.8 Hz), 5.42 (d, 1H, *J* = 17.2 Hz), 6.04 (m, 1H), 6.23 (d, 1H, *J* = 9.2 Hz), 6.80 (s, 1H), 6.84 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.36 (d, 1H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 69.4, 101.9, 112.8, 113.2, 113.3, 118.6, 128.9, 132.2, 143.5, 155.9, 161.2, 161.9.

7-((3-methylbut-2-en-1-yl)oxy)-2*H*-chromen-2-one (**39**, 91 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.75 (s, 3H), 1.79 (s, 3H), 4.56 (d, 2H, *J* = 6.8 Hz), 5.45 (t, 1H, *J* = 6.8 Hz), 6.22 (d, 1H, *J* = 9.6 Hz), 6.80 (d, 1H, *J* = 2.4 Hz), 6.83 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.34 (d, 1H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 18.4, 25.9, 65.5, 101.7, 112.5, 113.0, 113.3, 118.8, 128.8, 139.3, 143.5, 156.0, 161.4, 162.2.

7-(benzyloxy)-2*H*-chromen-2-one (**7**, 36% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 5.12 (s, 2H), 6.24 (d, 1H, *J* = 9.2 Hz), 6.87 (d, 1H, *J* = 2.0 Hz), 6.91 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.34 – 7.44 (m, 6H), 7.62 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ

(ppm) 70.6, 102.0, 112.8, 113.3 (2C), 127.6, 128.5 (2C), 128.8 (2C), 128.9, 135.9, 143.4, 155.9, 161.2, 162.0.

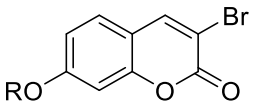
7-(2-hydroxyethoxy)-2*H*-chromen-2-one (**40**, 37% yield). ^{1}H-NMR (400 MHz, acetone-d_6): δ (ppm) 3.92 (t, 2H, *J* = 4.8 Hz), 4.19 (t, 2H, *J* = 4.8 Hz), 6.20 (d, 1H, *J* = 9.2 Hz), 6.87 (s, 1H), 6.91 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.55 (d, 1H, *J* = 8.8 Hz), 7.87 (d, 1H, *J* = 9.2 Hz). ^{13}C-NMR (100 MHz, Acetone-d_6): δ (ppm) 61.1, 71.3, 102.1, 113.5 (2C), 113.6, 130.1, 144.6, 156.8, 161.0, 163.3.

7-(3-bromopropoxy)-2*H*-chromen-2-one (**41**, 86% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.35 (quint, 2H, *J* = 6.0 Hz), 3.60 (t, 2H, *J* = 6.4 Hz), 4.17 (t, 2H, *J* = 5.6 Hz), 6.24 (d, 1H, *J* = 9.6 Hz), 6.82 (d, 1H, *J* = 2.0 Hz), 6.84 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.37 (d, 1H, *J* = 8.4 Hz), 7.63 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 29.6, 32.1, 66.1, 101.7, 112.9, 113.4 (2C), 129.0, 143.4, 156.0, 161.2, 162.0.

A2 Brominated ether derivatives

A 30% aqueous solution of H_2O_2 (3.0 eq) was added to the solution of ether derivatives of umbelliferone **3, 4, 35-37** (1 mmol) and NaBr (2.0 eq) in acetic acid (2.5 mL); then, the mixture was stirred at room temperature for 7-24 h depending on the substrate. The reaction was monitored by TLC. At the end, the crude was treated with Na_2SO_3 and extracted with EtOAc (3 x 10 mL). The reunited organic fractions were dried over anhydrous Na_2SO_4; after filtration, the solvent was evaporated under

reduced pressure. Final products **42-46** were isolated and purified by chromatographic column on silica gel with *n*-hexane/EtOAc.[44]



42: R = CH_3
43: R = C_2H_5
44: R = C_4H_9
45: R = C_8H_{17}
46: R = $C_{12}H_{25}$

3-bromo-7-methoxy-2*H*-chromen-2-one (**42**, 36% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.87 (s, 3H), 6.81 (s,1H), 6.86 (d, 1H, *J* = 8.4 Hz), 7.34 (d, 1H, *J* = 8.4 Hz), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 56.0, 100.9, 107.9, 113.2, 113.4, 128.2, 144.5, 155.3, 157.5, 163.2.

3-bromo-7-ethoxy-2*H*-chromen-2-one (**43**, 52% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.45 (t, 3H, *J* = 6.8 Hz), 4.08 (q, 2H, *J* = 6.8 Hz), 6.78 (d, 1H, *J* = 2.4 Hz), 6.84 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.32 (d, 1H, *J* = 8.4 Hz), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.6, 64.5, 101.4, 107.7, 113.2, 113.7, 128.2, 144.6, 155.2, 157.6, 162.6. HRMS (ESI) calcd for $C_{11}H_9BrNaO_3$ $[M+Na]^+$: 290.9633, found 290.9645.

3-bromo-7-butoxy-2*H*-chromen-2-one (**44**, 35% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.98 (t, 3H, *J* = 6.8 Hz), 1.50 (sext, 2H, *J* = 7.6 Hz), 1.80 (quint, 2H, *J* = 6.4 Hz), 4.01 (t, 2H, *J* = 6.4 Hz), 6.80 (s, 1H), 6.85 (d, 1H, *J* = 8.8 Hz), 7.32 (d, 1H, *J* = 8.8 Hz), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 13.9, 19.3, 31.1, 68.7, 101.4, 107.7,

113.0, 113.8, 128.2, 144.5, 153.3, 157.6, 162.8. HRMS (ESI) calcd for $C_{13}H_{13}BrO_3$ $[M+Na]^+$: 318.9946, found 318.9953.

3-bromo-7-(octyloxy)-2*H*-chromen-2-one (**45**, 37% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* = 6.4 Hz), 1.29 – 1.34 (m, 8H), 1.46 (quint, 2H, *J* = 6.4 Hz), 1.81 (quint, 2H, *J* = 6.8 Hz), 4.00 (t, 2H, *J* = 6.8 Hz), 6.79 (d, 1H, *J* = 2.0 Hz), 6.85 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.32 (d, 1H, *J* = 8.8 Hz), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.4, 23.2, 26.1, 29.1, 29.3, 29.4, 31.9, 69.2, 101.3, 107.9, 113.1, 113.8, 128.2, 144.6, 155.4, 157.6, 162.9. HRMS (ESI) calcd for $C_{17}H_{21}BrO_3$ $[M+Na]^+$: 375.0572, found 375.0586.

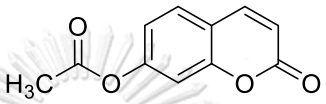
3-bromo-7-(dodecyloxy)-2*H*-chromen-2-one (**46**, 36% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.87 (t, 3H, *J* = 6.4 Hz), 1.26 – 1.31 (m, 16H), 1.45 (quint, 2H, *J* = 6.4 Hz), 1.80 (quint, 2H, *J* = 6.4 Hz, 2H), 4.00 (t, 2H, *J* = 6.4 Hz), 6.79 (d, 1H, *J* = 1.6 Hz), 6.85 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.32 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 26.1, 29.1, 29.4, 29.5, 29.6, 29.7, 29.7, 29.8, 32.0, 69.0, 101.4, 107.7, 113.0, 113.8, 128.2, 144.6, 155.3, 157.5, 162.7.

B Ester derivatives of umbelliferone

B1 Umbelliferyl acetate

Umbelliferone (**34**, 5 mmol) was stirred with acetic anhydride (4.0 eq) in ice bath and a catalytic amount of 96% H_2SO_4 (2 drops) was added dropwise. Then, 2.5 molar equivalents of Et_3N were added to the mixture and agitated for 10 min at room

temperature. After reaction was completed (analyzed by TLC), the crude was extracted with EtOAc (3 x 10 mL) and the combined extracts were washed with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue then was purified by column chromatography on silica gel with *n*-hexane/EtOAc to yield product **47.**[45]



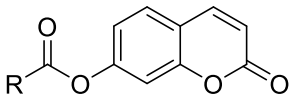
47: R = CH_3

2-oxo-2*H*-chromen-7-yl acetate (**47**, 99% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.33 (s, 3H), 6.38 (d, 1H, *J* = 9.6 Hz), 7.04 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.11 (d, 1H, *J* = 2.0 Hz), 7.48 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 10.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 21.2, 110.6, 116.2, 116.8, 118.5, 128.7, 142.9, 153.3, 154.8, 160.4, 168.8.

B2 Other derivatives

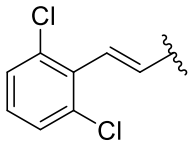
A mixture of PPh_3 (2 mmol) in 3mL CH_2Cl_2 and a mixture of carboxylic acid (1 mmol), Cl_3CCN (2 mmol) in 3mL CH_2Cl_2 were stirred at room temperature for approximately 1 h. Afterwards, a mixture of umbelliferone (**34**, 1 mmol) and 4-picoline (3 mmol) in 10 mL CH_2Cl_2 were added into previous mixture and refluxed at 38-40 ^{0}C for 6 h. Then, the organic layer was extracted with 10% HCl and saturated aqueous $NaHCO_3$, respectively. Furthermore, the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated using rotatory vacuum evaporator. The

products **48-53** were purified by subjecting to silica gel column with *n*-hexane/EtOAc. Products **54-55** were purified by washing with acetone.[46]

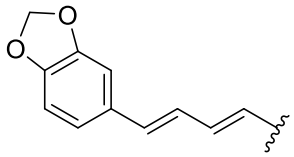


48: R = C_3H_7
49: R = C_5H_{11}
50: R = C_7H_{15}
51: R = C_9H_{19}
52: R = $C_{11}H_{23}$
53: R = $C_{15}H_{31}$

54: R =



55: R =



2-oxo-2*H*-chromen-7-yl butyrate (**48**, 35 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.04 (t, 3H, *J* = 7.6 Hz), 1.78 (m, 2H), 2.56 (t, 2H, *J* = 7.6 Hz), 6.37 (d, 1H, *J* = 9.6 Hz), 7.02 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.08 (d, 1H, *J* = 1.2 Hz), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 13.7, 18.4, 36.2, 110.5, 116.1, 116.7, 118.5, 128.6, 143.0, 153.4, 154.8, 160.4, 171.5.

2-oxo-2*H*-chromen-7-yl hexanoate (**49**, 30 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.92 (t, 3H, *J* = 6.8 Hz), 1.34 – 1.40 (m, 4H), 1.76 (quint, 2H, *J* = 7.6 Hz), 2.58 (t, 2H, *J* = 7.6 Hz), 6.38 (d, 1H, *J* = 9.6 Hz), 7.03 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.09 (d, 1H, *J* = 1.6 Hz), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.0, 22.4, 24.6, 31.3, 34.4, 110.6, 116.1, 116.7, 118.5, 128.6, 143.0, 153.4, 154.8, 160.4, 171.7.

2-oxo-2*H*-chromen-7-yl octanoate (**50**, 14 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* =6.4 Hz), 1.24 – 1.40 (m, 8H), 1.75 (quint, 2H, *J* = 7.6 Hz), 2.57 (t, 2H, *J* = 7.2 Hz), 6.37 (d, 1H, *J* = 9.6 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 7.08 (s, 1H), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 22.7, 24.9, 29.0, 29.1, 31.7, 34.4, 110.5, 116.1, 116.7, 118.5, 128.6, 143.0, 153.4, 154.8, 160.4, 171.7.

2-oxo-2*H*-chromen-7-yl decanoate (**51**, 46 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.87 (t, 3H, *J* = 6.4 Hz), 1.27 (m, 12H), 1.75 (quint, 2H, *J* = 7.6 Hz), 2.58 (t, 2H, *J* = 7.6 Hz), 6.38 (d, 1H, *J* = 9.6 Hz), 7.03 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.09 (d, 1H, *J* = 2.0 Hz), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 24.9, 29.2, 29.4 (2C), 29.5, 31.9, 34.5, 110.5, 116.1, 117.0, 118.5, 128.6, 143.0, 153.5, 154.8, 160.4, 171.7.

2-oxo-2*H*-chromen-7-yl dodecanoate (**52**, 38% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.87 (t, 3H, *J* = 6.0 Hz), 1.26 (m, 16H), 1.75 (quint, 2H, *J* = 7.2 Hz), 2.58 (t, 2H, *J* = 7.2 Hz), 6.38 (d, 1H, *J* = 9.2 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 7.10 (s, 1H), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 24.9, 29.2, 29.3, 29.4, 29.5, 29.7 (2C), 32.0, 34.5, 110.5, 116.1, 116.7, 118.5, 128.6, 143.0, 153.5, 154.8, 160.5, 171.7.

2-oxo-2*H*-chromen-7-yl palmitate (**53**, 73% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.87 (t, *J* = 6.4 Hz, 3H), 1.25 (m, 24H), 1.75 (quint, 2H, *J* = 7.2 Hz), 2.58 (t, 2H, *J*

= 7.6 Hz), 6.38 (d, 1H, *J* = 9.6 Hz), 7.04 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.10 (d, 1H, *J* = 2.0 Hz), 7.47 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 24.9, 29.2, 29.4, 29.5, 29.6, 29.7, 29.8 (2C), 29.8, 29.8 (2C), 32.0, 34.5, 110.6, 116.1, 116.7, 118.5, 128.6, 143.0, 153.5, 154.9, 160.4, 171.7.

2-oxo-2*H*-chromen-7-yl (*E*)-3-(2,6-dichlorophenyl) acrylate (**54**, 42% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 6.45 (d, 1H, *J* = 9.6 Hz), 6.88 (d, 1H, *J* = 16.4 Hz), 7.22 (d, 1H, *J* = 8.4 Hz), 7.29 (d, 2H, *J* = 7.2 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 7.56 (d, 1H, *J* = 8.4 Hz), 7.75 (d, 1H, *J* = 9.6 Hz), 8.06 (d, 1H, *J* = 16.4 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 110.6, 116.3, 116.9, 118.5, 125.3, 128.7, 129.1 (2C), 130.6, 131.5, 135.4 (2C), 141.1, 143.0, 153.5, 154.9, 160.4, 164.1. HRMS (ESI) calcd for $C_{18}H_{10}Cl_2O_4$ $[M+Na]^+$: 382.9854, found 382.9841.

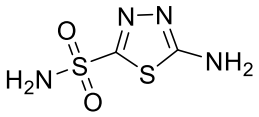
2-oxo-2*H*-chromen-7-yl(2*E*,4*E*)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoate (**55**, 45% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 6.01 (s, 2H), 6.12 (d, 1H, *J* = 15.2 Hz), 6.40 (d, 1H, *J* = 9.6 Hz), 6.76–7.04 (m, 4H), 7.04 (s, 1H), 7.12 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.18 (d, 1H, *J* = 1.2 Hz), 7.50 (d, 1H, *J* = 8.4 Hz), 7.63 (dd, 1H, *J* = 15.2, 4.0 Hz), 7.70 (d, *J* = 9.6 Hz, 1H). HRMS (ESI) calcd for $C_{21}H_{14}O_6$ $[M+K]^+$: 401.0428, found 401.0416.

2.2.3 Synthesis acetazolamide-based coumarins

2.2.3.1 Hydrolysis acetazolamide

To suspension of acetazolamide (**56**, 22 mmol), in EtOH was added conc. HCl 5 mL and reaction mixture was refluxed for 12 h. Solvent was evaporated, saturated

$NaHCO_3$ was poured slowly to the reaction mixture and compound was extracted by EtOAc. EtOAc layer was washed with brine and was dried over anhydrous Na_2SO_4, filtered and concentrated to obtained **57** in 61% yield.[47]

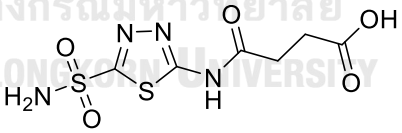


57

5-amino-1,3,4-thiadiazole-2-sulfonamide (**57**, 61% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 2H), 8.05 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.9, 171.6.

2.2.3.2 Synthesis acetazolamide derivative

To solution of **57** (3.0 mmol) in 4 mL acetonitrile (MeCN) was added succinic anhydride (3.3 mmol). The reaction mixture was heated at 100 °C for 12 h. After reaction completion, a white precipitate was filtered, wash with MeCN to get pure product **58**.[48]

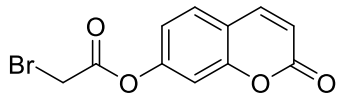


58

4-oxo-4-((5-sulfamoyl-1,3,4-thiadiazol-2-yl)amino)butanoic acid (**58**, 69% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (t, 2H, J = 6.8 Hz), 2.76 (t, 2H, J = 7.2 Hz), 8.32 (s, 2H), 13.06 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.3, 30.0, 161.1, 164.3, 171.5, 173.4.

2.2.3.3 Synthesis of umbelliferone derivative

The procedure of synthesis of ester **48-53** was applied to form ester **59**.



59

2-oxo-2H-chromen-7-yl 2-bromoacetate (**59**, 30% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.33 (s, 2H), 6.38 (d, 1H, *J* = 9.6 Hz), 7.04 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.10 (d, 1H, *J* = 2.0 Hz), 7.48 (d, 1H, *J* = 8.4 Hz), 7.68 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 21.2, 110.5, 116.2, 116.8, 118.5, 128.7, 143.0, 153.3, 154.8, 160.4, 168.8.

2.2.4 Bioassay

This experiment was conducted under the collaboration with Department of Microbiology, Faculty of Science, Chulalongkorn University. Yeast cells and test compounds were prepared in opaque-walled 96-well plates at the appropriate cell density for 10 µL. The test plate was incubated at room temperature for 30 min and then 10 µL of 20% (w/v) galactose was added into each well and incubated at 30 °C under an ambient atmosphere (low CO_2) or 5% (v/v) CO_2 condition (high CO_2) for the appropriate incubation time. A stock solution of 0.1 mg mL^{-1} resazurin sodium salt (Sigma Aldrich, USA) prepared in distilled water was added to each well to a final concentration of 0.03 mg mL−1 and further incubated at 30 °C in the dark until the color of the wells without the test compound changed from blue to pink, which

indicated the growth of yeast cells. The effective dose was defined as the concentration of the drug that could inhibit the growth of yeast cells and so prevent the color change of resazurin.[49] Reduction of resazurin to resorufin in yeast cell (with NADPH) was showed in **Figure 2.4**.

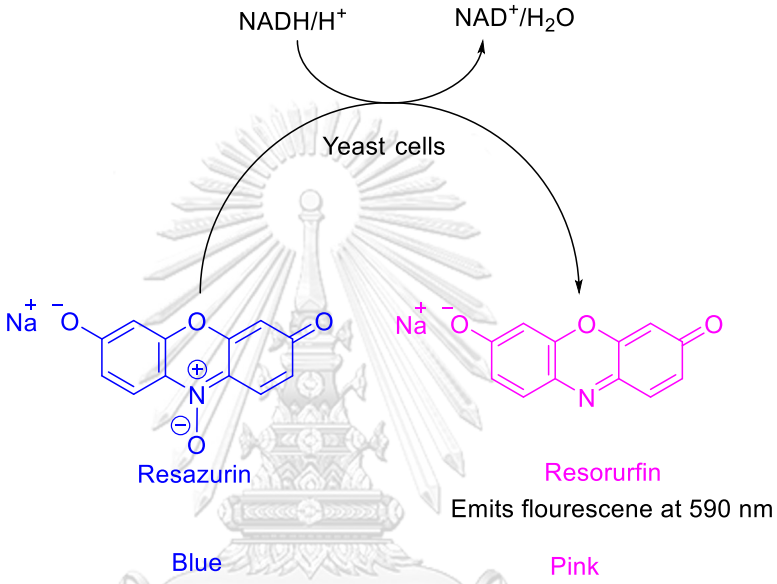


Figure 2.4 Reduction of resazurin to resorurfin in yeast cell (with NADH).[50]

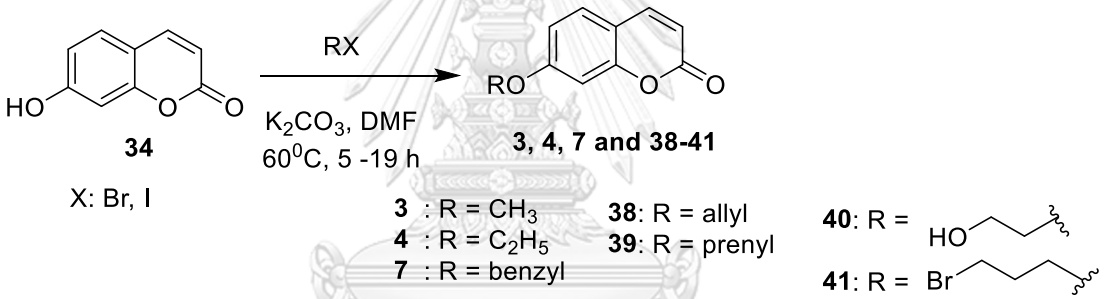
2.3 Results and discussion

2.3.1 Synthesis, structure elucidation and carbonic anhydrase II inhibition of coumarins

2.3.1.1 Ether and brominated ether derivatives of umbelliferone

In 2010, Maresca *et al*. reported that the synthesized series of ether derivatives of umbelliferone incorporating bulky chains (*n*-Pr, *n*-Bu, $PhCH_2$, $PhCH_2CH_2$) at position 7 decreased CA II inhibitory activity.[38] However, monosubstituted derivatives containing unsaturated side chain and saturated substituents containing other functional groups have not evaluated. Therefore, coumarins **38-41** were synthesized

and investigated (**Scheme 2.1**). Moreover, the ether derivatives of umbelliferone **3**, **4** and **7** were synthesized for comparison (**Scheme 2.1**). For known analogs, their structures were elucidated by comparing with reported NMR database.[43, 51-53] The ^{1}H and ^{13}C NMR spectra of **3**, **4**, **7**, **38**-**41** exhibited all feature signals of umbelliferone skeleton. Moreover, there were new signals derived from the side chain methyl, ethyl, allyl, prenyl, *etc.* consistent with the expected target molecules. Their ^{1}H and ^{13}C NMR interpretation are demonstrated in **Tables 2.1-2.4**. All compounds were tested against CA II and the results are presented in **Table 2.5**.



Scheme 2.1 Synthesis of ether derivatives **3**, **4**, **7** and **38**-**41**

Table 2.1 ^{1}H NMR chemical shift assignment of **3, 4, 38, 39**

Position	δ_H (ppm)			
	3	**4**	**38**	**39**
3	6.23 (d, 1H, *J* = 9.2 Hz)	6.22 (d, 1H, *J* = 9.6 Hz)	6.23 (d, 1H, *J* = 9.2 Hz)	6.22 (d, 1H *J* = 9.6 Hz)
4	7.62 (d, 1H, *J* = 9.6 Hz)	7.62 (d, 1H, *J* = 9.2 Hz)	7.62 (d, 1H, *J* = 9.6 Hz)	7.62 (d, 1H, *J* = 9.6 Hz)
5	7.36 (d, 1H, *J* = 8.8 Hz)	7.34 (d, 1H, *J* = 8.4 Hz)	7.36 (d, 1H, *J* = 8.4 Hz)	7.34 (d, 1H, *J* = 8.4 Hz)
6	6.83 (dd, 1H *J* = 8.8, 2.4 Hz)	6.81 (dd, 1H, *J* = 8.4, 2.4 Hz)	6.84 (dd, 1H, *J* = 8.4, 2.0 Hz)	6.83 (dd, 1H, *J* = 8.8, 2.4 Hz)
8	6.79 (d, 1H, *J* = 2.4 Hz)	6.78 (d, 1H, *J* = 2.4 Hz)	6.80 (s,1H)	6.80 (d, 1H, *J* = 2.4 Hz)
7-OR	methyl	ethyl	allyl	prenyl
	3.86 (s, 3H, H1′)	4.07 (q, 2H, *J* = 7.2 Hz, H1′), 1.44 (t, 3H, *J* = 7.2 Hz, H2′)	4.58 (d, 2H, *J* = 5.2 Hz, H1′), 5.98-6.08 (m, 1H, H2′), 5.32 (d, 1H, *J* = 10.8 Hz, H3a′), 5.42 (d, 1H, *J* = 17.2 Hz, H3b′)	4.56 (d, 2H, *J* = 6.8 Hz, H1′), 5.45 (t, 1H, *J* = 6.8 Hz, H2′), 1.75 (s, 3H, H4′) 1.79 (s, 3H, H4′)

Table 2.2 ^{13}C NMR chemical shift assignment of **3, 4, 38** and **39**

Position	δ_C (ppm)			
	3	**4**	**38**	**39**
2	161.3	161.3	161.2	161.4
3	112.7	113.0	113.2	113.0
4	143.5	143.5	143.5	143.5
5	128.9	128.8	128.9	128.8
6	113.2	113.0	113.6	113.3
7	163.0	162.3	161.9	162.2
8	101.0	101.4	101.9	101.7
9	156.0	156.0	155.9	156.0
10	112.7	112.5	112.8	112.5
7-OR	methyl	ethyl	allyl	prenyl
	55.9 (C1′)	64.5 (C1′), 14.7 (C2′)	69.4 (C1′), 132.2 (C2′), 118.6 (C3′)	65.5 (C1′), 118.8 (C2′), 139.3 (C3′), 18.4 (C4′), 25.9 (C4′)

Table 2.3 ^{1}H NMR chemical shift assignment of **7, 40** and **41**

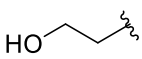
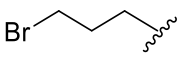
Position	δ_H (ppm)		
	7	**40**	**41**
3	6.24 (d, 1H, *J* = 9.2 Hz)	6.20 (d, 1H, *J* = 9.2 Hz)	6.24 (d, 1H, *J* = 9.6 Hz)
4	7.62 (d, 1H, *J* = 9.6 Hz)	7.87 (d, 1H, *J* = 9.2 Hz)	7.63 (d, 1H, *J* = 9.6 Hz)
5	7.34 – 7.44 (m, 1H)	7.55 (d, 1H, *J* = 8.8 Hz)	7.37 (d, 1H, *J* = 8.4 Hz)
6	6.91 (dd, 1H, *J* = 8.4, 2.4 Hz)	6.91 (dd, 1H, *J* = 8.8, 2.4 Hz)	6.84 (dd, 1H, *J* = 8.4, 2.4 Hz)
8	6.87 (d, 1H, *J* = 2.0 Hz)	6.87 (s, 1H)	6.82 (d, 1H, *J* = 2.0 Hz)
7-OR	benzyl		
	5.12 (s, 2H, H1′), 7.34 – 7.44 (m, 5H, H-Ar)	4.19 (t, 2H, *J* = 4.8 Hz, H1′), 3.92 (t, *J* = 4.8 Hz, 2H, H2′)	4.17 (t, 2H, *J* = 5.6 Hz, H1′), 2.35 (quint, 2H, J = 6.0 Hz, H2′), 3.60 (t, 2H, *J* = 6.4 Hz, H3′)

Table 2.4 ^{13}C NMR chemical shift assignment of **7, 40** and **41**

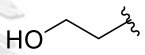
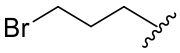
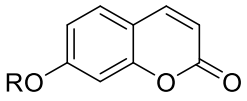
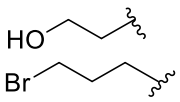
Position	δ_c (ppm)		
	7	**40**	**41**
2	161.2	161.0	161.2
3	113.3	113.5	112.9
4	143.4	144.6	143.4
5	127.6	130.1	129.0
6	113.3	113.6	113.4
7	162.0	163.3	162.0
8	102.0	102.1	101.7
9	155.9	156.8	156.0
10	112.8	113.5	113.4
7-OR	benzyl		
	70.6 (C1′), 128.8 (C2-Ar and C6-Ar), 128.5 (C3-Ar and C5-Ar), 128.9 (C4-Ar)	71.3 (C1′), 61.1 (C2′)	66.1 (C1′), 29.6 (C2′), 32.1 (C3′)

Table 2.5 Inhibition data of umbelliferone (**34**) and ether derivatives **3**, **4**, **7**, **38-41** and acetazolamide (AZA) against CA II.



34: R = H
3 : R = CH_3
4 : R = C_2H_5
38: R = allyl
39: R = prenyl
7 : R = benzyl
40: R =
41: R =

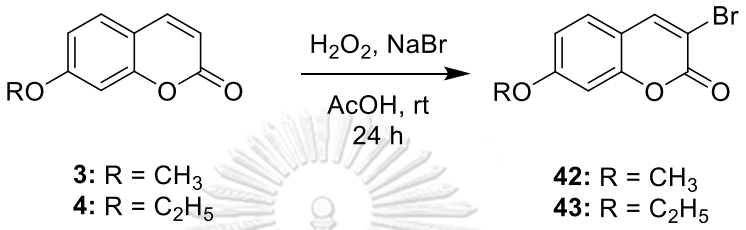


Entry	Compounds	ED_{50} (μM)	Toxicity (μM)
1	**34**	NA	
2	**3**	284	
3	**4**	263	
4	**38**	NA	
5	**39**	NA	
6	**7**	NA	
7	**40**	NA	
8	**41**	88	177
9	AZA	0.31	>25

NA: no activity

From **Table 2.5**, the inhibitory activities of **3** and **4** were not as good as previous reported in reference.[38] Furthermore, the results of **38** and **39** were not repeatable with the reported results of Ms. Samritsakuchal in 2016. However, the CA II inhibition data of these monosubstituted derivatives indicated that the substituents in side chain at position 7 may affect on the activity, especially, bromo substituent might improve the inhibitory activity (**41**, ED_{50} = 88 μM). This result encouraged to examine brominated ether derivatives. Firstly, two simple ether derivatives **3** and **4** were chosen to brominate (**Scheme 2.2**). For known **42**, the structure was elucidated by

comparing with reported NMR database.[54] The ^{1}H and ^{13}C NMR spectra of **42** and **43** (**Figures A17-A20**) exhibited all feature signals of umbelliferone skeleton. Moreover, the proton signal at position 3 disappeared because of the presence of bromo substitution. Their ^{1}H and ^{13}C NMR interpretation are depicted in **Table 2.6**.



Scheme 2.2 Synthesis of brominated ether derivatives **42**, **43**

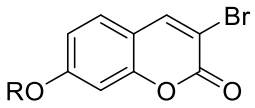
Table 2.6 ^{1}H and ^{13}C NMR chemical shift assignment of **42** and **43**

Position	42		43	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2		157.5		157.6
3		107.9		107.7
4	8.00 (s, 1H)	144.5	8.00 (s, 1H)	144.6
5	7.34 (d, 1H, *J* = 8.4 Hz)	128.2	7.32 (d, 1H, *J* = 8.4 Hz)	128.2
6	6.86 (d, 1H, *J* = 8.4 Hz)	113.4	6.84 (dd, 1H, *J* = 8.8, 2.4 Hz)	113.7
7		163.2		162.6
8	6.81 (s,1H)	100.9	6.78 (d, 1H, *J* = 2.4 Hz)	101.4
9		155.3		155.2
10		113.2		113.0
1′	3.87 (s, 3H)	56.0	4.08 (q, 2H, *J* = 6.8 Hz)	64.5
2′			1.45 (t, 3H, *J* = 6.8 Hz)	14.6

Two brominated ethers were evaluated for CA II inhibitory activity and the results are presented in **Table 2.7**. Interestingly, **43** containing ethoxy group

exhibited good activity to inhibit the enzyme (ED_{50} = 1.86 μM) while **42** with a methoxy group did not show any change in the inhibition comparing with ether **3**.

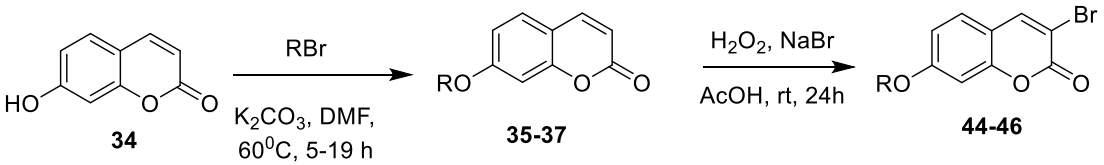
Table 2.7 Inhibition data of derivatives **42, 43** and AZA against CA II.



42: R = CH_3 **43**: R = C_2H_5

Entry	Compounds	ED_{50} (μM)
1	**42**	196
2	**43**	1.86
3	AZA	0.31

From the results, the CH_2 group of brominated ether derivatives illustrated an important role in CA II inhibition. Therefore, brominated ethers containing longer alkyl groups at position 7 may increase inhibitory activity. This hypothesis encouraged to synthesize more ethers and brominated derivatives (**Scheme 2.3**). Their ^1H and ^{13}C NMR characterization are presented in **Tables 2.8-2.11.**[43]



35: R = C_4H_9
36: R = C_8H_{17}
37: R = $C_{12}H_{25}$

44: R = C_4H_9
45: R = C_8H_{17}
46: R = $C_{12}H_{25}$

Scheme 2.3 Synthesis of ether derivatives **35-37** and brominated ether derivatives **44-46**

Table 2.8 ^{1}H NMR chemical shift assignment of **35-37**

Position	δ_H (ppm)		
	35	**36**	**37**
3	6.22 (d, 1H, *J* = 9.6 Hz)	6.23 (d, 1H, *J* = 9.6 Hz)	6.23 (d, 1H, *J* = 9.6 Hz)
4	7.62 (d, 1H, *J* = 9.6 Hz)	7.62 (d, 1H, *J* = 9.2 Hz)	7.62 (d, 1H, *J* = 9.6 Hz)
5	7.34 (d, 1H, *J* = 8.8 Hz)	7.35 (d, 1H, *J* = 8.4 Hz)	7.34 (d, 1H, *J* = 8.8 Hz)
6	6.82 (d, 1H, *J* = 8.8 Hz)	6.82 (dd, 1H, *J* = 8.4, 2.0 Hz)	6.82 (dd, 1H, *J* = 8.4, 2.0 Hz)
8	6.79 (s, 1H)	6.79 (s, 1H)	6.79 (d, 1H, *J* = 2.0 Hz)
7-OR	butyl	octyl	dodecyl
	4.00 (t, 2H, *J* = 6.8 Hz, H1′), 1.79 (quint, 2H, *J* = 6.4 Hz, H2′), 1.49 (sext, 2H, *J* = 7.6 Hz, H3′), 0.98 (t, 3H, *J* = 7.6 Hz, H4′)	4.00 (t, 2H, *J* = 6.8 Hz, H1′), 1.80 (quint, 2H, *J* = 6.8 Hz, H2′), 1.46 (quint, 2H, *J* = 6.8 Hz, H3′), 1.31 (m, 8H, H4′-H7′), 0.88 (t, 3H, *J* = 6.4 Hz, H8′)	4.00 (t, 2H, *J* = 6.4 Hz, H1′), 1.80 (quint, 2H, *J* = 6.8 Hz, H2′), 1.46 (quint, 2H, *J* = 6.4 Hz, H3′), 1.26 (m, 16H, H4′-H11′), 0.88 (t, 3H, *J* = 6.4 Hz, H12′)

Table 2.9 ^{13}C NMR chemical shift assignment of **35-37**

Position	δ_C (ppm)		
	35	**36**	**37**
2	161.4	161.4	161.4
3	113.0	113.1	113.1
4	143.5	143.5	143.5
5	128.8	128.8	128.8
6	113.1	113.0	113.1
7	162.6	162.6	162.6
8	101.5	101.5	101.5
9	156.1	156.1	156.1
10	112.5	112.5	112.5
7-OR	butyl	octyl	dodecyl
	68.5 (C1′), 31.1 (C-2′), 19.3 (C-3′), 13.9 (C-4′)	68.8 (C1′), 29.4 (C2′), 26.1 (C3′), 29.3 (C4′), 29.1 (C5′), 31.9 (C6′), 22.8 (C7′), 14.2 (C8′)	68.8 (C1′), 29.5 (C2′), 26.1 (C3′), 29.8, 29.8, 29.7, 29.7, 29.5, 29.1 (C4′-C9′), 32.0 (C10′), 22.8 (C11′), 14.2 (C12′)

Table 2.10 ^{1}H NMR chemical shift assignment of **44-46**

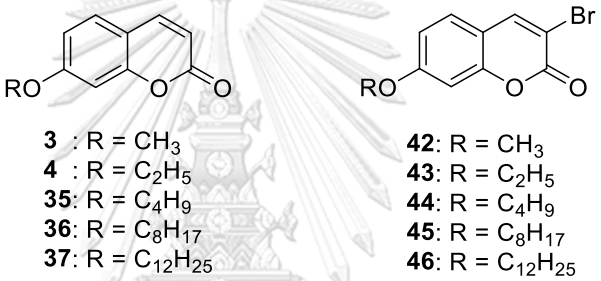
Position	δ_H (ppm)		
	44	**45**	**46**
4	8.00 (s, 1H)	8.00 (s, 1H)	8.00 (s, 1H).
5	7.32 (d, 1H, *J* = 8.8 Hz)	7.32 (d, 1H, *J* = 8.8 Hz)	7.32 (d, 1H, *J* = 8.4 Hz)
6	6.85 (d, 1H, *J* = 8.8 Hz)	6.85 (dd, 1H, *J* = 8.8, 2.4 Hz)	6.85 (dd, 1H, *J* = 8.8, 2.4 Hz)
8	6.80 (s, 1H)	6.79 (d, 1H, *J* = 2.0 Hz)	6.79 (d, 1H, *J* = 1.6 Hz)
7-OR	butyl	octyl	dodecyl
	4.01 (t, 2H, *J* = 6.4 Hz, H-1′), 1.80 (quint, 2H, *J* = 6.4 Hz, H2′), 1.50 (sext, 2H, *J* = 7.6 Hz, H3′), 0.98 (t, 3H, *J* = 6.8 Hz, H4′)	4.00 (t, 2H, *J* = 6.8 Hz, H-1′), 1.81 (quint, 2H, *J* = 6.8 Hz, H2′), 1.46 (quint, 2H, *J* = 6.8 Hz, H3′), 1.29-1.34 (m, 8H, H4′-H7′), 0.88 (t, 3H, *J* = 6.4 Hz, H8′)	4.00 (t, 2H, J = 6.4 Hz, H1′), 1.80 (quint, 2H, *J* = 6.4 Hz, H2′), 1.45 (quint, 2H, *J* = 6.4 Hz, H3′), 1.26-1.31 (m, 16H, H4’-H11′), 0.87 (t, 3H, *J* = 6.4 Hz, H12′)

Table 2.11 ^{13}C NMR chemical shift assignment of **44-46**

Position	δ_C (ppm)		
	44	**45**	**46**
2	157.6	157.6	157.6
3	107.7	107.9	107.7
4	144.5	144.6	144.6
5	128.2	128.2	128.2
6	113.8	113.8	113.8
7	162.8	162.9	162.8
8	101.4	101.3	101.4
9	153.3	155.4	155.3
10	113.0	113.1	113.0
7-OR	butyl	octyl	dodecyl
	68.7 (C1′), 31.1 (C-2′), 19.3 (C-3′), 13.9 (C-4′)	69.2 (C1′), 29.4 (C2′), 26.1 (C3′), 29.3 (C4′), 29.1 (C5′), 31.9 (C6′), 23.2 (C7′), 14.4 (C8′)	69.0 (C1′), 29.5 (C2′), 26.1 (C3′), 29.8, 29.7, 29.7, 29.6, 29.4, 29.1 (C4′-C9′), 32.0 (C10′), 22.8 (C11′), 14.2 (C12′)

All ether and brominated ether derivatives were evaluated against CA II. As presented in **Table 2.12**, brominated ethers containing long alkyl groups did not improve the inhibitory activity as expectation. Among this series, only a new compound **43** showed the best activity. However, when **43** was retested, the inhibitory activity was not the same as the first time, this compound inhibited CA II at 186 μM and showed toxicity with the enzyme at this concentration.

Table 2.12 Inhibition data of derivatives **3, 4, 35-37**, **42-46** and AZA against CA II.



3 : R = CH_3
4 : R = C_2H_5
35: R = C_4H_9
36: R = C_8H_{17}
37: R = $C_{12}H_{25}$

42: R = CH_3
43: R = C_2H_5
44: R = C_4H_9
45: R = C_8H_{17}
46: R = $C_{12}H_{25}$

Entry	Compounds	ED_{50}[a] (μM)	ED_{50}[b] (μM)	Toxicity (μM)
1	**3**	284		
2	**4**	263		
3	**35**	NA		
4	**36**	NA		
5	**37**	NA		
6	**42**	196		
7	**43**	1.86	186	186
8	**44**	NA		
9	**45**	NA		
10	**46**	NA		
11	**AZA**	0.31		

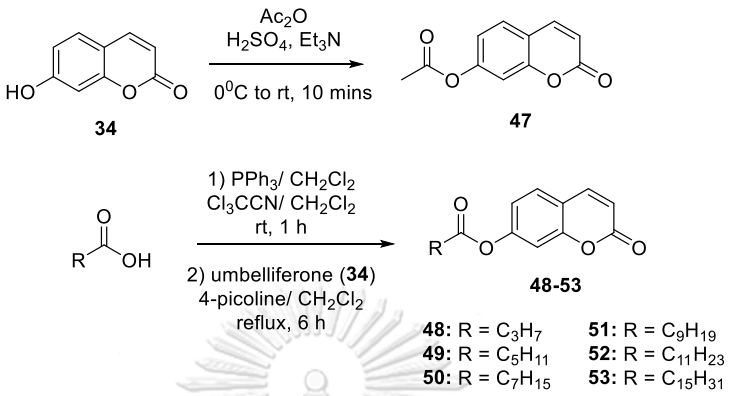
[a] First screening; [b] Second screening

NA: no activity

2.3.1.2 Ester derivatives of umbelliferone

Umbelliferone containing a hydroxy group at C-7 provided a good possibility for further modification such as esterification. According to Ms. Samritsakuchal's research in 2016, umbelliferyl octanoate showed excellent inhibitory activity against CA II with MED < 0.032 μM. Moreover, umbelliferyl dodecanoate and umbelliferyl hexadecanoate decreased activity to inhibit the enzyme. These results suggested that long chain carbon of ester derivatives reduce CA II inhibition. However, in 2018, De Luca *et al*. reported that umbelliferyl acetate was ineffective towards CA II.[1] Therefore, more esters containing alkyl chain at position 7 (such as C_3H_7, C_5H_{11}, C_9H_{19}) should be synthesized and tested for CA II inhibitory activity (**Scheme 2.4**). In addition, umbelliferyl acetate, octanoate, dodecanoate and hexadecanoate were also synthesized following the previous reported protocols (**Scheme 2.4**) and characterized by ^{1}H and ^{13}C NMR. The ^{1}H NMR spectra of **47-53** showed the signals of 7-substituted coumarin group containing four high intensity doublet signals and a doublet of doublet signal in the range of 6.37-7.68 ppm together with the singlet signal of methyl group of acetate, triplet signals of methyl group of other esters, methylene protons adjacent to carbonyl carbon, and a multiplet signal of methylene groups. The ^{1}H NMR spectra assignment of **47-53** is assembled in **Tables 2.13** and **2.14**. Furthermore, the ^{13}C NMR spectra showed the signals of nine carbons of coumarin scaffold and the signals of carbonyl and carbon in side chain at position 7. For known analogs, their structures were elucidated by comparing with reported NMR

database.[55] The ^{13}C NMR spectra assignment of **47-53** is gathered in **Tables 2.15** and **2.16.**



Scheme 2.4 Synthesis of ester derivatives **47-53**

Table 2.13 ^{1}H NMR chemical shift assignment of **47-50**

Position	δ_H (ppm)			
	47	**48**	**49**	**50**
3	6.38 (d, 1H, *J* = 9.6 Hz)	6.37 (d, 1H, *J* = 9.6 Hz)	6.38 (d, 1H, *J* = 9.6 Hz)	6.37 (d, 1H, *J* = 9.6 Hz)
4	7.68 (d, 1H, *J* = 10.2 Hz)	7.68 (d, 1H, *J* = 9.6 Hz)	7.68 (d, 1H, *J* = 9.6 Hz)	7.68 (d, 1H, *J* = 9.6 Hz)
5	7.48 (d, 1H, *J* = 8.4 Hz)	7.47 (d, 1H, *J* = 8.4 Hz)	7.47 (d, 1H, *J* = 8.4 Hz)	7.47 (d, 1H, J = 8.4 Hz)
6	7.04 (dd, 1H, *J* = 8.4, 1.6 Hz)	7.02 (dd, 1H, *J* = 8.4, 2.0 Hz)	7.03 (dd, 1H, *J* = 8.4, 2.0 Hz)	7.02 (d, 1H, *J* = 8.4Hz)
8	7.11 (d, 1H, *J* = 2.0 Hz)	7.08 (d, 1H, *J* = 1.2 Hz)	7.09 (d, 1H, *J* = 1.6 Hz)	7.08 (s, 1H)
7-OCOR	CH_3	C_3H_7	C_5H_{12}	C_7H_{15}
	2.33 (s, 3H, H1′)	2.56 (t, 2H, *J* = 7.6 Hz, H1′), 1.78 (m, 2H, H2′), 1.04 (t, 3H, *J* = 7.6 Hz, H3′)	2.58 (t, 2H, *J* = 7.6 Hz, H1′), 1.76 (quint, 2H, *J* = 7.6 Hz, H2′), 1.34-1.40 (m, 4H, H3′ and H4′), 0.92 (t, 3H, *J* = 6.8 Hz, H5′)	2.57 (t, 2H, *J* = 7.2 Hz, H1′), 1.75 (quint, 2H, *J* = 7.6 Hz, H2′), 1.24-1.40 (m, 8H, H3′-H6′), 0.92 (t, 3H, *J* = 6.8 Hz, H7′)

Table 2.14 ^{1}H NMR chemical shift assignment of **51-53**

Position	δ_H (ppm)		
	51	**52**	**53**
3	6.38 (d, 1H, *J* = 9.6 Hz)	6.38 (d, 1H, *J* = 9.2 Hz)	6.38 (d, 1H, *J* = 9.6 Hz)
4	7.68 (d, 1H, *J* = 9.6 Hz)	7.68 (d, 1H, *J* = 9.6 Hz)	7.68 (d, 1H, *J* = 9.2 Hz)
5	7.47 (d, 1H, *J* = 8.4 Hz)	7.47 (d, 1H, *J* = 8.4 Hz)	7.47 (d, 1H, *J* = 8.4 Hz)
6	7.03 (dd, 1H, *J* = 8.4, 2.4 Hz)	7.04 (d, 1H, *J* = 8.4 Hz)	7.04 (dd, 1H, *J* = 8.4, 2.0 Hz)
8	7.09 (d, 1H, J = 2.0 Hz)	7.10 (s, 1H)	7.10 (d, 1H, *J* = 2.0 Hz)
7-OCOR	C_9H_{19}	$C_{11}H_{23}$	$C_{15}H_{31}$
	2.58 (t, 2H, *J* = 7.6 Hz, H1′), 1.75 (quint, 2H, *J* = 7.6 Hz, H2′), 1.27 (m, 12H, H3′-H8′), 0.87 (t, 3H, *J* = 6.4 Hz, H9′)	2.58 (t, 2H, *J* = 7.2 Hz, H1′), 1.75 (quint, 2H, *J* = 7.2 Hz, H2′), 1.26 (m, 16H, H3′-H10′), 0.87 (t, 3H, *J* = 6.0 Hz, H11′)	2.58 (t, 2H, *J* = 7.2 Hz, H1′), 1.75 (quint, 2H, *J* = 7.2 Hz, H2′), 1.25 (m, 24H, H3′-H14′), 0.87 (t, 3H, *J* = 6.4 Hz, H15′)

Table 2.15 ^{13}C NMR chemical shift assignment of **47-50**

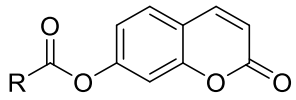
Position	δ_C (ppm)			
	47	**48**	**49**	**50**
2	160.4	160.4	160.4	160.4
3	116.2	116.0	116.1	116.1
4	142.9	143.0	143.0	142.9
5	128.7	128.6	128.6	128.6
6	118.5	118.5	118.5	118.5
7	154.8	154.8	154.8	154.8
8	110.5	110.5	110.5	110.5
9	153.3	153.4	153.4	153.4
10	116.8	116.7	116.7	116.7
7-OCOR	CH_3	C_3H_7	C_5H_{11}	C_7H_{15}
	168.8 (CO), 21.2 (C2′),	171.5 (CO), 36.2 (C2′), 18.4 (C3′), 13.7 (C4′)	171.7 (CO), 34.4 (C2′), 31.3 (C3′), 24.6 (C4′), 22.4 (C5′), 13.9 (C6′)	171.7 (CO), 34.4 (C2′), 31.7 (C3′), 29.1 (C4′), 29.0 (C5′), 24.9 (C6′), 22.7 (C7′), 14.1 (C8′)

Table 2.16 ^{13}C NMR chemical shift assignment of **51-53**

Position	δ_C (ppm)		
	51	**52**	**53**
2	160.4	160.5	160.4
3	116.1	116.1	116.1
4	143.0	143.0	143.0
5	128.6	128.6	128.6
6	118.5	118.5	118.5
7	154.9	154.8	154.9
8	110.5	110.5	110.6
9	153.5	153.4	153.5
10	116.7	116.7	116.7
7-OCOR	C_9H_{19}	$C_{11}H_{23}$	$C_{15}H_{31}$
	171.7 (CO), 34.5 (C2′), 31.9 (C3′), 29.5 (C4′), 29.4 (C5′, C6′), 29.2 (C7′), 24.9 (C8′), 22.8 (C9′), 14.2 (C10′)	171.7 (CO), 34.5 (C2′), 32.0 (C3′), 29.7 (C4′, C5′), 29.5 (C6′), 29.4 (C7′), 29.3 (C8′), 29.2 (C9′), 24.9 (C10′), 22.8 (C11′), 14.2 (C12′)	171.7 (CO), 34.5 (C2′), 32.0 (C3′), 29.8 (C4′, C5′), 29.8 (C6′), 29.8 (C7′, C8′), 29.7 (C9′), 29.6 (C10′), 29.5 (C11′), 29.4 (C12′), 29.2 (C13′), 24.9 (C14′), 22.8 (C15′), 14.2 (C16′)

Coumarin derivatives were submitted to test for antihuman CA II and are illustrated in **Table 2.17**. For the first time, the acetate of umbelliferone **47** showed effective activity at ED_{50} 2.45 μM, but this result was also extremely different from literature. De Luca *et al*. reported that this derivative did not inhibit CA II with K_I < 200 μM.[1] In addition, the results were not repeatable in case of ester **50, 52**.

Table 2.17 Inhibition data of derivatives **47-53** and AZA against CA II.



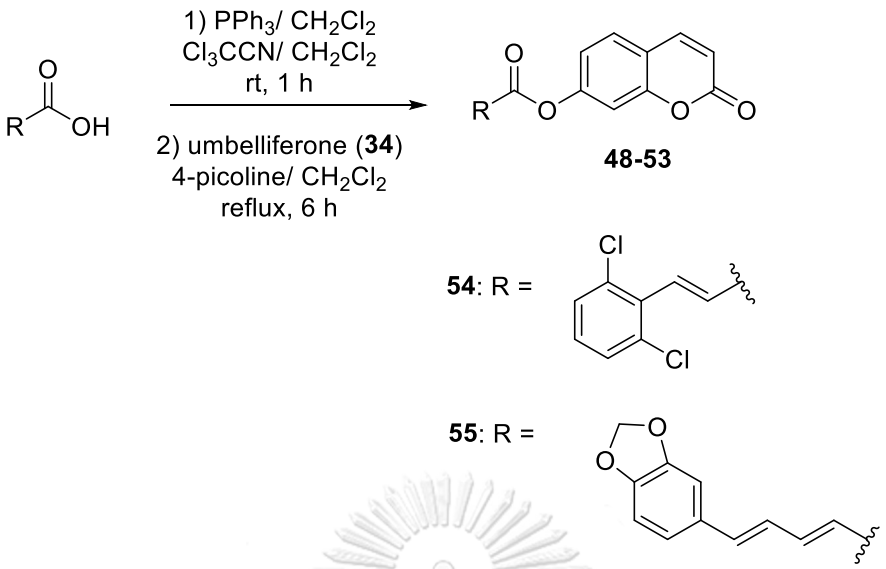
47: R = CH_3 **48**: R = C_3H_7 **49**: R = C_5H_{11} **50**: R = C_7H_{15} **51**: R = C_9H_{19} **52**: R = $C_{11}H_{23}$ **53**: R = $C_{15}H_{31}$

Entry	Compounds	ED_{50}[a] (μM)	ED_{50}[b] (μM)	ED_{50}[c] (μM)	Toxicity (μM)
1	**47**	2.45	NA	NA	
2	**48**	NA			
3	**49**	192			
4	**50**	174	NA	43	173
5	**51**	NA			
6	**52**	14.5	NA	NA	
7	**53**	125			
8	AZA	0.31			>25

[a] First screening; [b] Second screening; [c] Third screening

NA: no activity

Moreover, other ester derivatives of 2,6-dichlorocinnamic and piperic acid were synthesized (**Scheme 2.5**). These two new esters were characterized by ^{1}H and ^{13}C NMR and confirmed by high resolution mass spectra (HRMS). The spectra assignment of **54** and **55** are assembled in **Tables 2.18** and **2.19.** Two esters were evaluated for CA II inhibitory activity and the results are presented in **Table 2.20**.



Scheme 2.5 Synthesis of ester derivatives **54**, **55**

Table 2.18 ^{1}H NMR chemical shift assignment of **54** and **55**

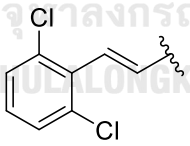
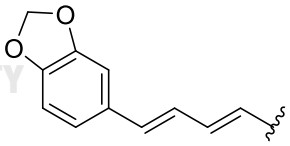
Position	δ_H (ppm)	
	54	**55**
3	6.45 (d, 1H, *J* = 9.6 Hz)	6.40 (d, 1H, *J* = 9.6 Hz)
4	7.75 (d, 1H, *J* = 9.6 Hz)	7.70 (d, 1H, *J* = 9.6 Hz)
5	7.56 (d, 1H, *J* = 8.4 Hz)	7.50 (d, 1H, *J* = 8.4 Hz)
6	7.29 (d, 1H, *J* = 7.2 Hz)	7.12 (dd, 1H, *J* = 8.4, 1.6 Hz)
8	7.29 (s, 1H)	7.18 (d, 1H, *J* = 1.2 Hz)
7-OCOR		
	8.06 (d, 1H, *J* = 16.4 Hz, H1′), 6.88 (d, 1H, *J* = 16.4 Hz, H2′), 7.44 (d, 2H, *J* = 8.0 Hz, H3-Ar and H5-Ar), 7.22 (d, 1H, *J* = 8.4 Hz, H4-Ar)	6.12 (d, 1H, *J* = 15.2 Hz, H1′), 7.63 (dd, 1H, *J* = 15.2, 4.0 Hz, H2′), 6.76-7.04 (m, 4H, H3′, H4′, H5-Ar and H6-Ar), 7.04 (s, 1H, H2-Ar), 6.01 (s, 2H, O-CH_2-O)

Table 2.19 ^{13}C NMR chemical shift assignment of **54**

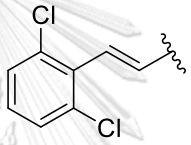
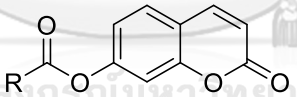
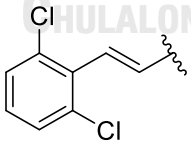
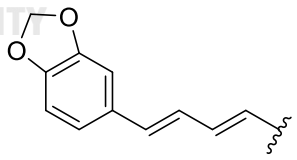
Position	δ_C (ppm)
	54
2	160.5
3	116.3
4	143.0
5	128.7
6	118.5
7	164.1
8	110.6
9	154.9
10	116.9
7-OCOR	 153.4 (CO), 125.3 (C2′), 130.6 (C3′), 131.5 (C1-Ar), 141.1 (C2-Ar, C6-Ar), 129.1 (C3-Ar, C5-Ar), 135.4 (C4-Ar)

Table 2.20 Inhibition data of **54** and **55** and AZA against CA II.



54: R = 

55: R = 

Entry	Compounds	ED_{50}[a] (μM)	ED_{50}[b] (μM)
1	**54**	13.8	NA
2	**55**	NA	
3	AZA	0.31	

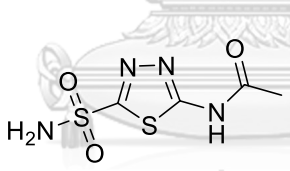
[a] First screening; [b] Second screening

NA: no activity

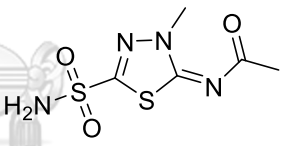
Ester **54** showed effective inhibitory activity at 13.8 μM while **55** did not inhibit CA II. However, when **54** was retested, this compound was not effective against the enzyme.

2.3.2 Synthesis, structure elucidation and carbonic anhydrase II inhibition of acetazolamide-based coumarins

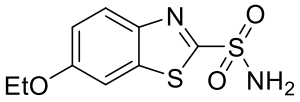
Sulfonamide carbonic anhydrase inhibitors have a firm place in medicine, mainly as antiglaucoma or antisecretory drugs, diuretics, as well as agents for the treatment/prevention of several neurological disorders. There are four systemic sulfonamide drugs used clinically, mainly as antiglaucoma agents for a long time: acetazolamide (**55**), methazolamide (**60**), ethoxzolamide (**61**), and dichlorophenamide (**62**).[56]



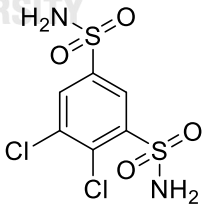
56



60



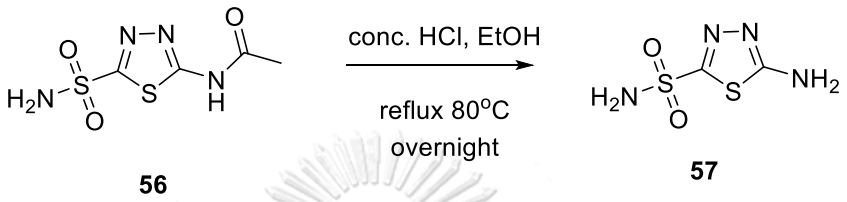
61



62

However, these systematically acting inhibitors showed side effect because they inhibited all the physiologically relevant CA isozymes.[20] From this side effect, the combination of sulfonamide (such as AZA) and coumarin might improve the

selectivity of CAIs. Therefore, AZA-based coumarins would be model compounds. First, AZA was hydrolyzed to form **57** (**Scheme 2.6**). The hydrolysis was successfully with 61% yield. The ^{1}H and ^{13}C NMR assignment of hydrolyzed product **57** are illustrated in **Table 2.21**.



56 **57**

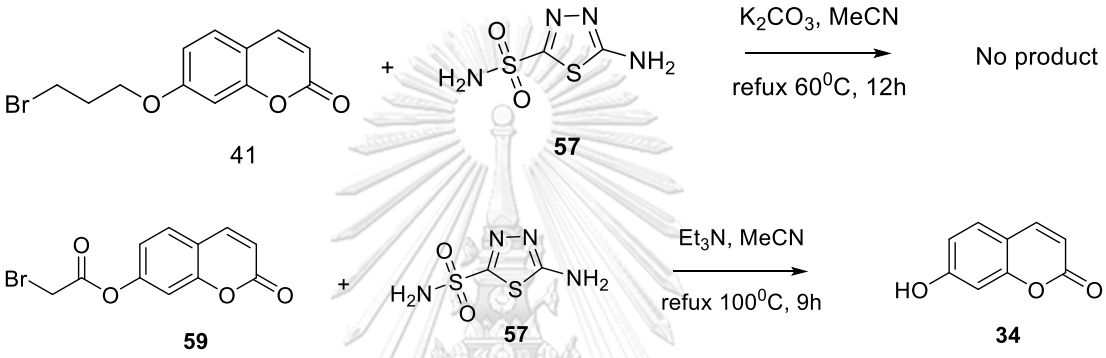
Scheme 2.6 Synthesis of hydrolyzed acetazolamide

Table 2.21 ^{1}H and ^{13}C NMR chemical shift assignment of **57**

Position	57	
	δ_H (ppm)	δ_C (ppm)
H_2N-SO_2	8.05 (s, 2H)	
NH_2	7.80 (s, 2H)	
C-SO_2-NH_2		171.5
C-NH_2		158.0

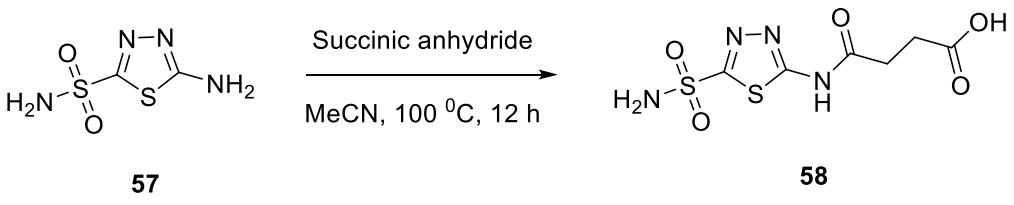
Hydrolyzed AZA **57** was used to react with ether derivative of umbelliferone **41** by Williamson reaction. A mixture of **41** (1.5 mmol), **57** (3 eq), and K_2CO_3 (3 eq) in MeCN (10.0 mL) was stirred at 60 ^{0}C for 12 h (**Scheme 2.7**). However, the reaction was not occurred, no product was observed. In this case, K_2CO_3 might not activate hydrolyzed AZA. Therefore, another base Et_3N was replaced K_2CO_3 in the reaction of ester derivative of umbelliferone **59.** An amount of 0.1 mmol of ester **59** and 0.1 mmol of hydrolyzed AZA **57** were dissolved in 1 mL of MeCN, and Et_3N (1 eq) was

added under stirring. The reaction was stirred at 100 ^{0}C for 9 h and created umbelliferone (**34**) (**Scheme 2.7**). Ester derivative **59** was not stable under base condition at high temperature, so it reversed to starting material **34**. From the result, hydrolyzed product of AZA did not work as good nucleophile, thereby AZA-based coumarins were not further examined.



Scheme 2.7 Reactions of hydrolyzed AZA with ether derivative **41** and ester derivative **59**

According to literature review, hydrolyzed product of AZA reacted with succinic anhydride (**Scheme 2.8**). Therefore, the derivative of AZA **58** was prepared, characterized and assigned in **Table 2.22**.

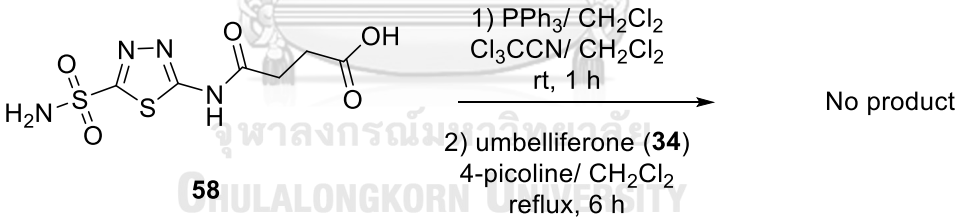


Scheme 2.8 Synthesis of derivative of AZA **58**

Table 2.22 ^{1}H and ^{13}C NMR chemical shift assignment of **58**

Position	58	
	δ_H (ppm)	δ_C (ppm)
2		171.6
5		173.4
1′		161.2
2′	2.60 (t, 2H, *J* = 6.8 Hz)	28.3
3′	2.76 (t, 2H, *J* = 7.2 Hz)	30.0
4′		164.2
-NH_2	8.32 (s, 2H)	

Derivative **58** containing acid group was used to react with umbelliferone (**34**), PPh_3, Cl_3CCN and 4-picoline in CH_2Cl_2 according to previous procedure (**Scheme 2.9**). However, **58** was difficult to dissolve in CH_2Cl_2, so it was not changed to acid chloride leading to fail reaction.



Scheme 2.9 Reaction of derivative of AZA **58**

2.4 Conclusion

The inhibition data of umbelliferone derivatives was not repeatable for ether, brominated ether and ester derivatives of umbelliferone. Therefore, AZA-based coumarins were not be a target for further study and other activities should be investigated. Moreover, coumarins have been reported to exhibit anticoagulant, anti-tumor or antiviral properties whereas other derivatives behave as enzyme inhibitors

or display antioxidant or anti-inflammatory properties.[57] Many coumarins were investigated on α-glucosidase inhibitors such as biscoumarins, substituted coumarins, hydroxycoumarin derivatives and sulfonamide coumarins.[58-62] Therefore, α-glucosidase inhibitiory activity is a good candidate for further examination in this research.

CHAPTER 3

SYNTHESIS OF COUMARINS AS α-GLUCOSIDASE INHIBITORS

3.1 Introduction

3.1.1 Diabetes mellitus

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the produced insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.[63]

Diabetes can lead to complications in many parts of the body such as stroke, blindness, heart attack, kidney failure, amputation and increase the risk of dying prematurely. The World Health Organization (WHO) reported that the number of diabetics was about 422 million in 2016. That was 1 person in 11. In this year, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. In 2016, WHO evaluates that diabetes was the seventh leading cause of death. After one year, approximately 425 million adults (20-79 years) were living with diabetes and this will rise to 629 million by 2045 according to International Diabetes Federation (IDF). IDF Diabetes Atlas Eighth edition 2017 is illustrated in **Figure 3.1.**

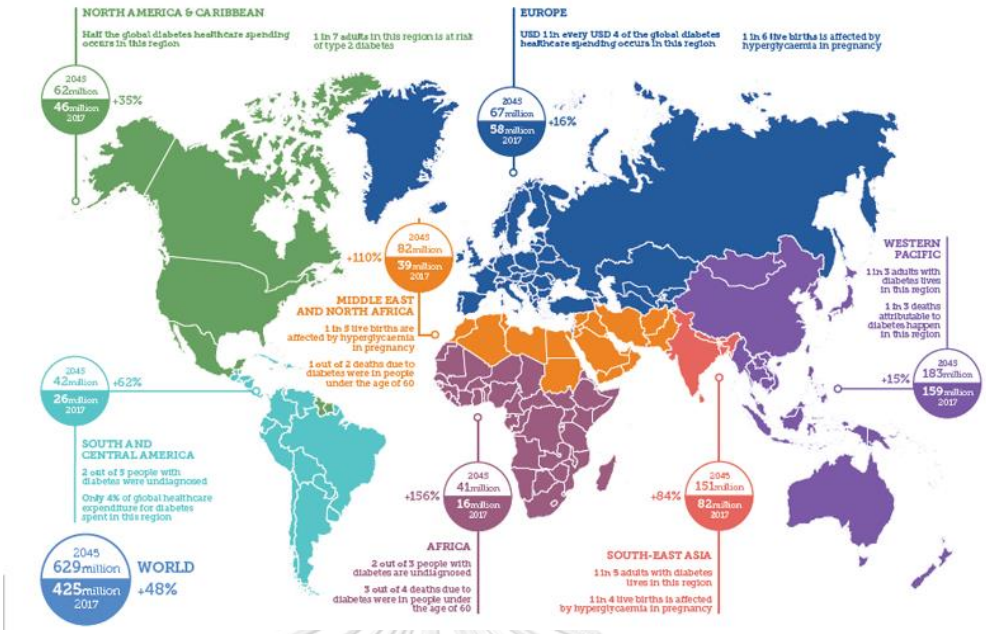


Figure 3.1 IDF Diabetes Atlas Eighth edition 2017 (www.idf.org)

Diabetes can be classified into two major varieties, types 1 and 2, and several minor variants.[63]

Type 1 diabetes, formerly called juvenile-onset diabetes, which accounts for only 5–10 % of those with diabetes, previously encompassed by terms insulin-dependent diabetes.[64] Type 1 diabetes tends to develop quickly, early in life. Relatively well-defined genetic factors interact with undefined environmental cues to cause the body to attack its own insulin-producing cells—the islet cells, or beta cells, of the pancreas in type 1 diabetes. This type lacks islets cells, so the diabetics must inject themselves with insulin several times a day to maintain their blood sugar concentrations within a healthy range.[63]

However, type 2 diabetes, previously referred to as non-insulin-dependent diabetes, accounts for 90–95% of cases of diabetes. Compared to type 1, type 2

generally follows a much more gradual progression, developing in middle age. This type relates to obesity, lack of physical activity, and unhealthy diets. Obesity leads to insulin resistance, where fat and muscle cells become less responsive to insulin and less able to take up glucose. In response, the pancreas ramps up insulin production, but eventually it is unable to compensate for insulin resistance and the concentration of blood glucose increases out of control. Type 2 diabetics cannot produce enough insulin. During the later stages of type 2 diabetes, the pancreas appears to wear out and the patient becomes dependent on injected insulin to maintain blood glucose concentrations.[63] Diet and exercise are the first steps in the treatment of type 2 diabetes. But if these measurements alone fail to effectively control blood glucose levels, starting oral drug therapy is recommended.[65] Type 1 and type 2 diabetes are presented in **Figure 3.2**.

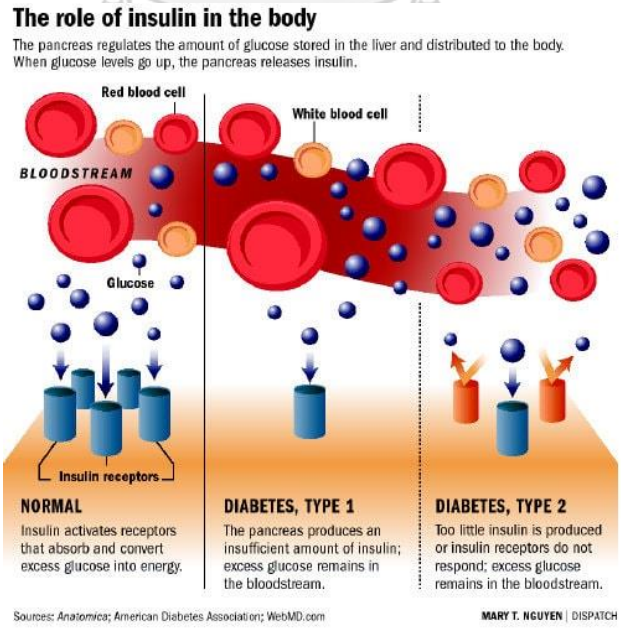


Figure 3.2 Type 1 and type 2 diabetes (www.diarystore.com)

3.1.2 α-glucodidase inhibitors

3.1.2.1 Acarbose as α-glucosidase inhibitors

Carbohydrases often consume as starch, glycogen (both polysaccharides), and sucrose (disaccharide) must be digested into monosaccharides before they can be absorbed. Six classes of oral glucose-lowering drugs used exclusively for treatment or prevention of type 2 diabetes mellitus drugs have been available: biguanides (metformin), sulfonylurea (*e.g.* tolbutamide), glinidines (*e.g.* repaglinide), thiazolidinediones (*e.g.* pioglitazone), dipeptidyl peptidase IV inhibitors (*e.g.* sitagliptin) and α-glucosidase inhibitors (AGIs; *e.g.* acarbose).[66] α-Glucosidase, located on the surface of the small intestinal microvilli, is responsible for digesting polysaccharides, oligosaccharides, and disaccharides into monosaccharides.[67, 68] AGIs reversibly inhibit α-glucosidase, consequently delaying the absorption of sugars from the gut, so reducing glucose uptake and the resulting post-prandial hyperglycemia observed in diabetes.[65, 69] Acarbose is the most widely prescribed AGI.[65] Acarbose, a peusdo tetrasaccharide of microbial origin (*Actinoplanes*), is made of two glucose residues linked *via* an amino sugar and α-1,4-glycosidic bonds to one unsaturated cyclitol residue.[68, 70] Intramolecular nitrogen of acarbose is responsible for the high affinity to the carbohydrate binding site of various α-glucosidase.[68, 71] Acarbose mechanism action is presented in **Figure 3.3.**

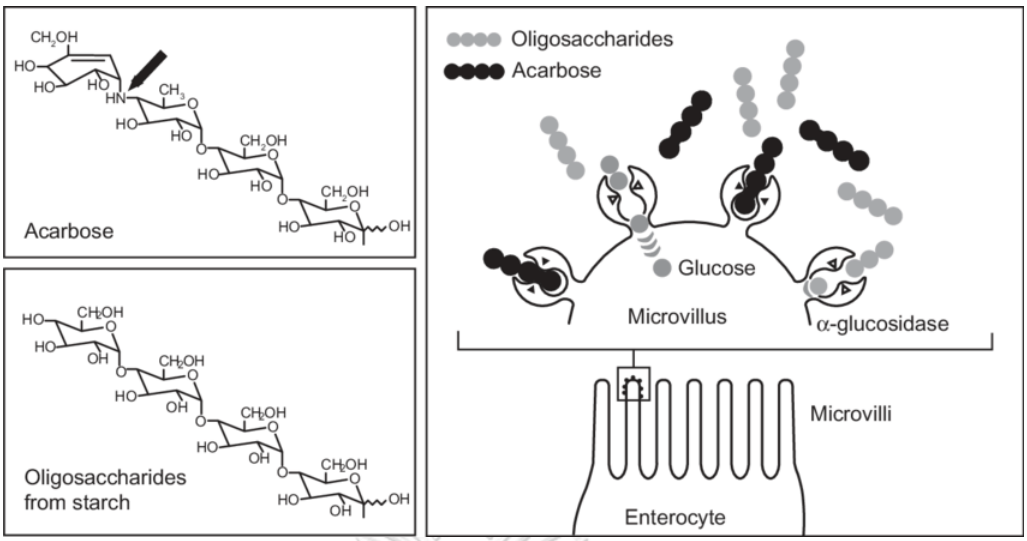


Figure 3.3 Acarbose mechanism action: Competitive inhibition of acarbose toward intestinal enzymatic hydrolysis of oligosaccharides.[72]

However, acarbose has low efficacy with high IC_{50} values against the enzyme. Using acarbose caused some side effects such as flatulence, abdominal cramp, vomiting and diarrhea. However, acarbose, when carefully prescribed in combination with adequate dietary advice, seems to be one of the safest antidiabetic agents available, used either alone or in combination with other glucose-lowering strategies.[73] To discover better safety and efficacy AGIs, scientist have been continuous further studied with diversed compounds including coumarin scaffold.

*3.1.2.2 Coumarins as **α**-glucosidase inhibitors*

Several coumarins have synthesized and evaluated against **α**-glucosidase such as biscoumarin[58, 59], chromenone[60], hydroxycoumarin[61], and sulfonamide coumarin derivatives.[62] Chemical structures of these AGIs were illustrated in **Figure 3.4**.

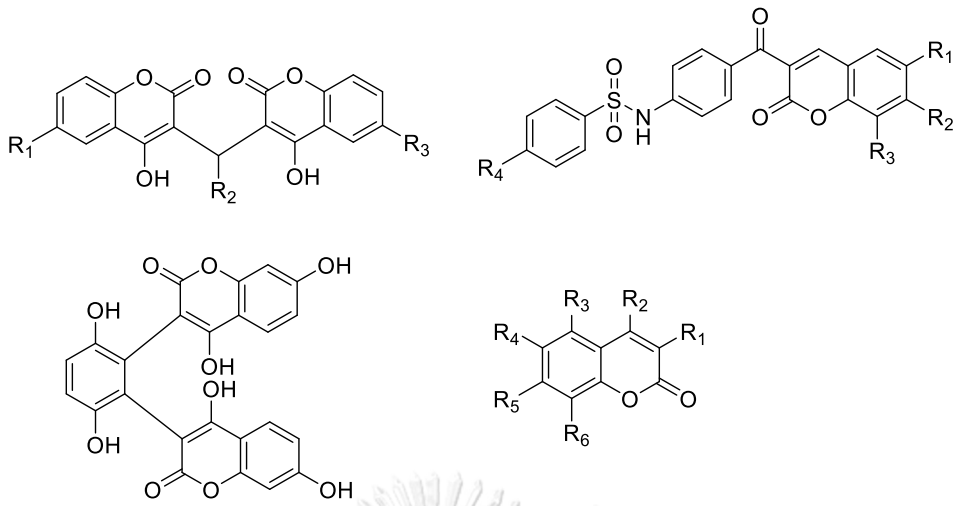


Figure 3.4 Chemical structures of some α-glucosidase inhibitors containing coumarin moieties.[59-62]

Umbelliferone is a benzopyrone widely found in edible fruits, golden apple (*Aegle marmelos* Correa) and fruits of bitter orange (*Citrus aurantium*). Moreover, umbelliferone was reported to significantly reduce glucose in STZ-diabetic rats.[74]

3.1.2.3 Aim of this study

In this study, derivatives of umbelliferone (ethers, brominated ethers, esters, sulfonamide coumarins and sulfonate coumarins) were synthesized, characterized and evaluated for inhibitory activity against α-glucosidase.

3.2 Experimental section

3.2.1 General procedure

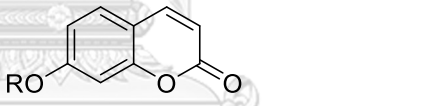
^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, acetone-d_6 or DMSO-d_6 using a Bruker Ultrashield 400 Plus NMR spectrometer or a Varian Mercury NMR spectrometer with an Oxford YH400 magnet operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. High

resolution mass spectra (HRMS) were recorded on a Bruker Daltonics microTOF using electron spray ionization (ESI).

All solvents used in this research were distilled prior to use except those which were reagent grades. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 PF254). Merck silica gel (No. 7734) was used as stationary phase on open column chromatography.

3.2.2 Synthesis of ether derivatives of umbelliferone

Excluding to ether derivatives of umbelliferone synthesized in chapter 2, two additional compounds were synthesized according to the same previous method as described in chapter 2.



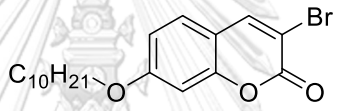
63: R = $C_{10}H_{21}$ **64**: R = HO

7-(decyloxy)-2*H*-chromen-2-one (**63**, 99 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* = 6.4 Hz), 1.27 (m, 12H), 1.44-1.47 (m, 2H), 1.78-1.84 (m, 2H), 4.00 (t, 2H, *J* = 6.4 Hz), 6.23 (d, 1H, *J* = 9.6 Hz), 6.80 (s, 1H), 6.83 (d, 1H, *J* = 8.8 Hz), 7.35 (d, 1H, *J* = 8.8 Hz), 7.63 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 26.1, 29.1, 29.4, 29.4, 29.6 (2C), 32.0, 68.8, 101.5, 112.5, 113.1, 113.2, 128.8. 143.5, 156.1, 161.4, 162.6.

7-(3-hydroxypropoxy)-2*H*-chromen-2-one (**64**, 60% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.05 (m, 2H), 3.84 (t, 2H, *J* = 6.0 Hz), 4.14 (t, 2H, *J* = 6.4 Hz), 6.20 (d, 1H, *J* = 9.2 Hz), 6.77 (d, 1H, *J* = 2.0 Hz), 6.81 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.33 (d, 1H, *J* = 8.4 Hz), 7.60 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 31.9, 59.5, 65.8, 101.5, 112.7, 113.0 (2C), 128.9, 143.6, 155.9, 161.5, 162.3.

3.2.3 Synthesis of a brominated ether derivative of umbelliferone

The ether derivative of umbelliferone **63** was brominated with the same procedure as mentioned for previous brominated ethers in chapter 2.



65

3-bromo-7-(decyloxy)-2*H*-chromen-2-one (**65,** 51% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.88 (t, 3H, *J* = 6.4 Hz), 1.27 (m, 12H), 1.46 (t, 2H, *J* = 7.2 Hz), 1.80 (quint, 2H, *J* = 6.4 Hz), 4.00 (t, 2H, *J* = 6.4 Hz), 6.79 (d, 1H, *J* = 2.0 Hz), 6.85 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.32 (d, 1H, *J* = 8.8 Hz), 8.00 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 22.8, 26.1, 29.1, 29.4, 29.4, 29.6 (2C), 32.0, 69.0, 101.4, 107.7, 113.0, 113.8, 128.1. 144.6, 155.3, 157.6, 162.8. HRMS (ESI) calcd for $C_{19}H_{25}BrO_3$ $[M+Na]^+$: 403.0884, found: 403.0861.

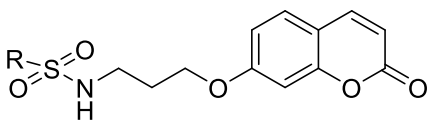
3.2.4 Synthesis of sulfonamide coumarins

3.2.4.1 Preparation of sulfonamide alkyl bromides

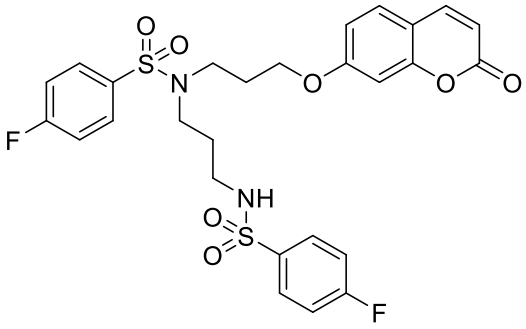
Selected arylsulfonyl chloride (4 mmol) and 3-bromopropylamine hydrobromide (4.6 mmol) were suspended in anhydrous DCM (14 mL) under N_2 atmosphere. The reaction mixture was cooled to 0 ℃ in an ice-bath and then treated dropwise with Et_3N (1.34 mL, 9.6 mmol) over a period of 10 min. The reaction mixture was stirred for 10 min under ice-cooling, then diluted with DCM (50 mL) and washed with HCl 2M (2 x 60 mL) and brine (2 x 60 mL). The organic solvent was evaporated at reduced pressure after drying over anhydrous Na_2SO_4 to yield sulfonamide alkyl bromides.[75]

3.2.4.2 Synthesis of sulfonamide coumarins from sulfonamide alkyl bromides

A mixture of umbelliferone (**34**, 2 mmol), sulfonamide alkyl bromide (1 eq), and K_2CO_3 (2 eq) in DMF (5.0 mL) was stirred at 55–60 ^{0}C for 13 h. The reaction mixture was extracted with EtOAc, and the combined extracts were washed with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue then was purified by column chromatography on silica gel with *n*-hexane/EtOAc to yield **66-70.**[43]



66: R = C_6H_5
67: R = C_6F_5
68: R = 4-Cl-C_6H_4
69: R = 4-F-C_6H_4



70

N-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)benzenesulfonamide (**66**, 38% yield). ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 1.84 (t, 2H, *J* = 6.4 Hz), 2.93 (br, 2H), 4.04 (t, 2H, *J* = 5.6 Hz), 6.27 (d, 1H, *J* = 9.2 Hz), 6.88-6.86 (m, 1H), 7.61-7.55 (m, 4H), 7.71 (br, 1H), 7.80-7.78 (m, 2H), 7.96 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 28.6, 65.4 (2C), 101.2, 112.3, 112.4, 112.7, 126.4, 129.2, 129.4, 132.3, 140.4, 144.3, 155.3, 160.3, 161.6. HRMS (ESI) calcd for $C_{18}H_{17}NO_5S$ $[M+Na]^+$: 382.0725, found 382.0722.

2,3,4,5,6-pentafluoro-*N*-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)benzene-sulfonamide (**67**, 37% yield). ^{1}H-NMR (400 MHz, acetone-d_6): δ (ppm) 2.24 (quint, 2H, *J* = 7.6 Hz), 4.04 (t, 4H, *J* = 7.6 Hz), 6.36 (d, 1H, *J* = 9.6 Hz), 7.14 (s, 1H), 7.25 (d, 1H, *J* = 8.4 Hz), 7.77 (d, 1H, *J* = 8.8 Hz), 7.99 (d, 1H, *J* = 9.6 Hz). ^{13}C-NMR (100 MHz, Acetone-d_6): δ (ppm) 15.8, 52.2 (2C), 104.4, 113.6, 116.0, 116.7, 131.1, 144.1, 156.5, 159.9, 160.3.

4-chloro-*N*-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)benzenesulfonamide (**68**, 25% yield). ^{1}H-NMR (400 MHz, acetone-d_6): δ (ppm) 2.01-1.96 (m, 2H), 3.18 (t, 2H, *J* = 6.0 Hz), 4.11 (t, 2H, *J* = 6.0 Hz), 6.21 (d, 1H, *J* = 9.6 Hz), 6.79 (s, 1H), 7.56-7.54 (m, 3H), 7.85

(d, 2H, *J* = 8.4 Hz), 7.89 (d, 1H, *J* = 9.6 Hz, 1H). ^{13}C-NMR (100 MHz, acetone-d_6): δ (ppm) 40.7, 66.5 (2C), 113.7 (2C), 129.5 (2C), 130.1, 130.1 (2C), 138.8, 140.9, 145.0, 156.8, 161.2, 163.0. HRMS (ESI) calcd for $C_{18}H_{16}ClNO_5S$ $[M+Na]^+$: 416.0335, found 416.0332.

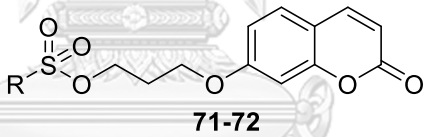
4-fluoro-*N*-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)benzenesulfonamide (**69**, 27% yield). ^{1}H-NMR (400 MHz, acetone-d_6): δ (ppm) 2.02-1.97 (m, 2H), 3.16 (q, 2H, *J* = 6.0 Hz), 4.14 (t, 2H, *J* = 6.0 Hz), 6.21 (d, 1H, *J* = 9.2 Hz), 6.71 (s, 1H), 6.81 (s, 1H), 6.86 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.31 (m, 2H), 7.56 (d, 1H, *J* = 8.8 Hz), 7.94-7.88 (m, 3H). ^{13}C-NMR (100 MHz, acetone-d_6): δ (ppm) 40.6, 66.4 (2C), 102.1, 113.5, 113.6, 113.7, 116.8, 117.0, 130.1, 130.6, 130.7, 144.6, 156.9, 161.0, 163.0, 164.4, 166.9. HRMS (ESI) calcd for $C_{18}H_{16}FNO_5S$ $[M+Na]^+$: 400.0631, found 400.0636.

4-fluoro-*N*-(3-((4-fluorophenyl)sulfonamido)propyl)-N-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)benzenesulfonamide (**70**, 12% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.81 (t, 2H, *J* = 5.6 Hz), 2.02 (t, 2H, *J* = 5.6 Hz), 3.04 (br, 2H), 3.24 (t, 2H, *J* = 6.4 Hz), 3.28 (t, 2H, *J* = 6.8 Hz), 3.97 (t, 2H, *J* = 5.6 Hz), 5.29 (s, 1H), 6.26 (d, 1H, *J* = 9.6 Hz), 6.71 (s, 1H), 6.78 (dd, 1H, *J* = 8.8, 2.0 Hz), 7.17 (q, 4H, *J* = 8.4 Hz), 7.36 (d, 1H, *J* = 8.8 Hz), 7.63 (d, 1H, *J* = 9.6 Hz), 7.80 (dd, 2H, *J* = 8.8, 5.2 Hz), 7.87 (dd, 2H, *J* = 8.4, 4.8 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 28.7, 29.1, 29.8, 39.9, 46.3, 46.3, 65.5, 101.6, 112.8, 112.9, 113.5, 116.4, 116.6, 116.6, 129.0, 129.8, 129.9, 129.9, 130.0, 143.5, 155.9, 161.2,

161.8, 163.9, 164.0, 166.5, 166.6. HRMS (ESI) calcd for $C_{27}H_{26}F_2N_2O_7S_2$ $[M+Na]^+$: 615.1047, found 615.1049.

3.2.5 Synthesis of sulfonate coumarins

Into a flask equipped with a stirrer and a cooling tube, **64** (0.6 mmol), aryl sulfonyl chloride (3.3 eq), toluene (1.2 mL), 2 mmol of Et_3N as a base. The mixture was stirred at room temperature for 20 h. After stirring, the reaction solution was extracted with EtOAc, and the combined extracts were washed with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue then was purified by column chromatography on silica gel with *n*-hexane/EtOAc to yield **71-72.**[76]



71-72

71: R = -C_6H_5
72: R = 4-F-C_6H_4

3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl benzenesulfonate (**71**, 97% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.16 (quint, 2H, *J* = 5.6 Hz), 4.02 (t, 2H, *J* = 5.6 Hz), 4.26 (t, 2H, *J* = 6.0 Hz), 6.24 (d, 1H, *J* = 9.2 Hz), 6.66 (s, 1H), 6.72 (d, 1H, *J* = 8.8 Hz), 7.34 (d, 1H, *J* = 8.8 Hz), 7.48 (t, 2H, *J* = 7.2 Hz), 7.58 (t, 1H, *J* = 7.2 Hz), 7.63 (d, *J* = 9.6 Hz), 7.88 (d, 2H, *J* = 8.0 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 28.8, 63.9, 67.0, 101.7, 112.6, 112.9, 113.4, 127.9 (2C), 128.9 (2C), 129.4, 133.9, 136.0, 143.4, 155.9, 161.2, 161.7. HRMS (ESI) calcd for $C_{18}H_{16}O_6S$ $[M+Na]^+$: 383.0565, found 383.0573.

3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl 4-fluorobenzenesulfonate (**72**, 57% yield). ^{1}H-NMR (400 MHz, Acetone-d_6): δ (ppm) 2.18 (quint, 2H, *J* = 6.0 Hz), 4.12 (t, 2H, *J* = 6.0 Hz), 4.32 (t, 2H, *J* = 6.0 Hz), 6.22 (d, 1H, *J* = 9.2 Hz), 6.77 (d, 1H, *J* = 2.4 Hz), 6.81 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.33 (d, 2H, *J* = 8.8 Hz), 7.55 (d, 1H, *J* = 8.8 Hz), 7.89 (d, 1H, *J* = 9.6 Hz), 7.99 (m, 2H). ^{13}C-NMR (100 MHz, Acetone-d_6): δ (ppm) 64.8, 68.4 (2C), 102.1, 113.3, 113.5, 113.7, 113.8, 117.4, 117.6, 130.1, 131.7, 131.8, 144.5, 156.8, 160.9, 162.7, 165.3, 167.8. HRMS (ESI) calcd for $C_{18}H_{15}FO_6S$ $[M+Na]^+$: 401.0471, found 401.0448.

3.2.6 **α**-Glucosidase inhibitory activity test

α-Glucosidase (0.1 μM/mL) and substrate (1 mM *p*-nitrophenyl-**α**-*D*-glucopyranoside, *p*-NPG) were dissolved in 0.1 M phosphate buffer, pH 6.9, 10 μL of synthesized compound (1 mg/mL in DMSO) was pre-incubated with 40 μL of **α**-glucosidase at 37 ºC for 10 min. A 50 μL substrate solution was then added to the reaction mixture and incubated at 37 ºC for 20 min and terminated by adding 100 μL of 1 M Na_2CO_3. The enzymatic hydrolysis of the *p*-NPG was monitored based on the amount of *p*-nitrophenol released into the reaction mixture (**Figure 3.5**). The enzymatic activity was quantified by measuring the absorbance at 405 nm (Bio-Rad microplate reader model 3550 UV). The percentage inhibition was calculated by [(A0-A1)/A0] × 100, where A0 is the absorbance without the sample, and A1 is the absorbance with the sample. The IC_{50} value was determined from a plot of percentage inhibition *versus* sample concentration. Acarbose was used as standard control and the experiment was performed in duplicate.[77]

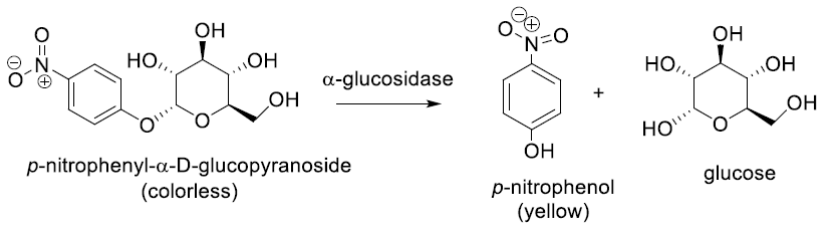


Figure 3.5 Hydrolysis of *p*-NPG by **α**-glucosidase

3.3 Results and discussion

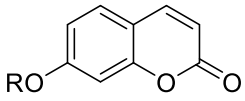
3.3.1 Ether derivatives of umbelliferone

Umbelliferone (**34**) and ether derivatives **3**, **4**, **7**, **35-37, 40**, **41** and **64** were tested against **α**-glucosidase. The inhibition data is illustrated in **Table 3.1**. Umbelliferone (**34**) and ether derivatives **3**, **4**, **7**, **35-37, 40**, **41** and **64** were tested against **α**-glucosidase. The inhibition data is illustrated in **Table 3.1**. **3** comprising a methoxy group showed the same activity as **36** and **37, 4** and **35** bearing ethoxy and butoxy groups demonstrated less activities (IC_{50} >200 μM). The increasing of alkyl chain length of the ether analogs of umbelliferone (entries 2-5) causes these derivatives more hydrophobic. The increasing of the hydrophobicity of compound resulted in increasing the activity until eight carbon atoms (entry 4). However, the coumarin ether containing twelve carbon atoms (entry 5) in side chain exhibiting less activity, indicated that the compound containing too hydrophobic part did not display good anti-**α**-glucosidase activity.

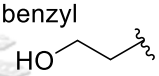
Other alkyl chains with unsaturation of ether derivatives **7**, **38**, **39** and three ether derivatives of umbelliferone containing -OH and -Br **40**, **41** and **64** were chosen to test for **α**-glucosidase inhibition. All six compounds are not promising candidates

for this activity (IC_{50} > 200 μM). These derivatives did not improve the compound potency to inhibit this enzyme.

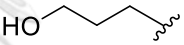
Table 3.1 Inhibition data of ether derivatives **3**, **4**, **7**, **35-41 64**, umbelliferone (**34**) and acarbose against **α**-glucosidase



34: R = H
3 : R = CH_3
4 : R = C_2H_5
35: R = C_4H_9
36: R = C_8H_{17}
37: R = $C_{12}H_{25}$

38: R = allyl
39: R = prenyl
7 : R = benzyl
40: R = 

41: R = 

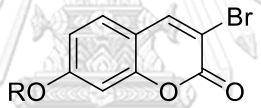
64: R = 

Entry	Compounds	IC_{50} (μM)
1	**3**	146.2 ± 1.13
2	**4**	>200
3	**35**	>200
4	**36**	149.5 ± 1.57
5	**37**	158.4 ± 0.90
6	**38**	>200
7	**39**	>200
8	**7**	>200
9	**40**	>200
10	**41**	>200
11	**64**	>200
12	**34**	>200
13	acarbose	93.63 ± 0.49

3.3.2 Brominated ether derivatives of umbelliferone

In 1970, Lien *et al*. reported the role of hydrophobic interactions in inhibiting the relatively specific enzymatic reactions of five enzyme systems: lipoxygenase, *D*-amino acid oxidase, hydroxyindole-*O*-methyltransferase, carbonic anhydrase, monoamine oxidase.[78] The α-glucosidase inhibitory activity of ether derivatives of umbelliferone from the previous study indicated that the hydrophobic interaction affected on the activity. To evaluate this hypothesis, brominated ethers **42-46** were chosen to test for α-glucosidase inhibition. The results are accumulated in **Table 3.2**.

Table 3.2 Inhibition data of brominated ether derivatives **42-46** and acarbose against α-glucosidase.



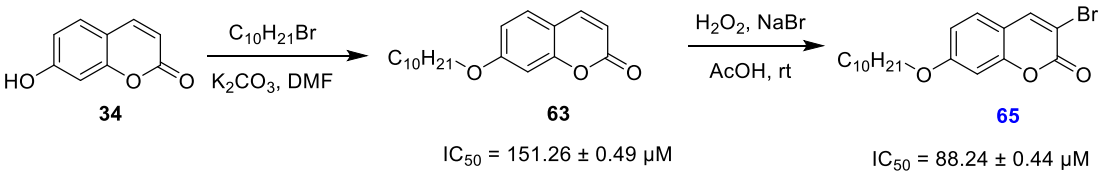
42: R = CH_3 **43**: R = C_2H_5 **44**: R = C_4H_9 **45**: R = C_8H_{17} **46**: R = $C_{12}H_{25}$

Entry	Compounds	IC_{50} (μM)
1	**42**	>200
2	**43**	>200
3	**44**	160.7 ± 0.93
4	**45**	68.0 ± 1.30
5	**46**	99.05 ± 0.42
6	Acarbose	93.63 ± 0.49

In general, brominated umbelliferones showed a significant increase inhibitory activity compared to parent ether derivatives. In this work, the longer alkyl chain of

the ether analogs of brominated umbelliferones, the better activity towards anti-**α**-glucosidase was observed possibly due to more hydrophobicity of synthesized compounds. The increasing of the compound hydrophobicity resulted in enhancing the activity until eight carbon atoms. Conversely, for aliphatic substituent containing twelve carbon atoms exhibiting decreased activity, indicated that a hydrophobic–hydrophilic balance was required. Prior investigation by Janoff and coworkers, named this phenomenon as a ‘‘cut off effect” in which the higher alkyl chain lengths will cause a decrease activity due to the limitation of membrane partition coefficient (lipid/aqueous). The solubility of lipid increased at a rate faster than the change in membrane partition coefficient by the increasing of alkyl chain lengths. For higher chain lengths, there is a limitation of partition coefficient affecting on the concentration at the site of action inadequate for having a substantial effect on the membrane of **α**-glucosidase.[79]

To prove the hydrophobic effect, ether derivative **63** and brominated ether derivative **65** of umbelliferone containing ten carbon atoms were prepared (**Scheme 3.1**). The ^{1}H and ^{13}C NMR assignment of these derivatives are reported in **Table 3.3**. Inhibition data of these derivatives (**Figure 3.6**) verified the hydrophobic effect in inhibitory activity against **α**-glucosidase where **63** and **65** showed IC_{50} of 151.26±0.49 and 88.24±0.44 μM, respectively.



Scheme 3.1 Synthesis of ether derivatives **63** and brominated ether derivative **65**

Table 3.3 ^{1}H and ^{13}C NMR chemical shift assignment of **63** and **65**

Position	63		65	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2		161.4		157.6
3	6.23 (d, 1H, *J* = 9.6 Hz)	113.1		107.7
4	7.63 (d, 1H, *J* = 9.6 Hz)	143.5	8.00 (s, 1H)	144.6
5	7.35 (d, 1H, *J* = 8.8 Hz)	128.8	7.32 (d, 1H, *J* = 8.8 Hz)	128.1
6	6.83 (d, 1H, *J* = 8.8 Hz)	113.1	6.85 (dd, 1H, *J* = 8.4, 2.4 Hz)	113.8
7		162.6		162.8
8	6.80 (s,1H)	101.5	6.79 (d, 1H, *J* = 2.0 Hz)	101.4
9		156.1		155.3
10		113.0		113.0
7-O-$C_{10}H_{21}$	4.00 (t, 2H, *J* = 6.4 Hz, H1′), 1.78-1.84 (m, 2H, H2′), 1.44-1.47 (m, 2H, H3′), 1.27 (m, 12H, H4′ - H9′), 0.88 (t, 3H, *J* = 6.4 Hz, H10′)	68.8 (C1′), 32.0 (C2′), 29.6 (C3′, C4′), 29.6 (C4′), 29.4 (C5′), 29.4 (C6′), 29.1 (C7′), 26.1 (C8′), 22.8 (C9′), 14.2 (C10′)	4.00 (t, 2H, *J* = 6.4 Hz, H1′), 1.80 (quint, 2H, J = 6.4 Hz, H2′), 1.46 (m, 2H, H3′), 1.27 (m, 12H, H4′- H9′), 0.88 (t, 3H, *J* = 6.4 Hz, H10′)	69.0 (C1′), 32.0 (C2′), 29.6 (C3′), 29.6 (C4′), 29.4 (C5′), 29.4 (C6′), 29.1 (C7′), 26.1 (C8′), 22.8 (C9′), 14.2 (C10′)

Moreover, it was clear that bromo substituent at position 3 of ether derivatives of umbelliferone improved expressively the inhibitory activity. While ether derivatives

of umbelliferone **35-37**, **63** inhibited **α**-glucosidase with IC_{50} >200, 150, 158, 151 μM, their brominated derivatives **44-46**, **65** significant increased effectivities to 161, 68, 99, 88 μM respectively. **α**-Glucosidase inhibition of ether, brominated ether derivatives of umbelliferone and acarbose is illustrated in **Figure 3.6**.

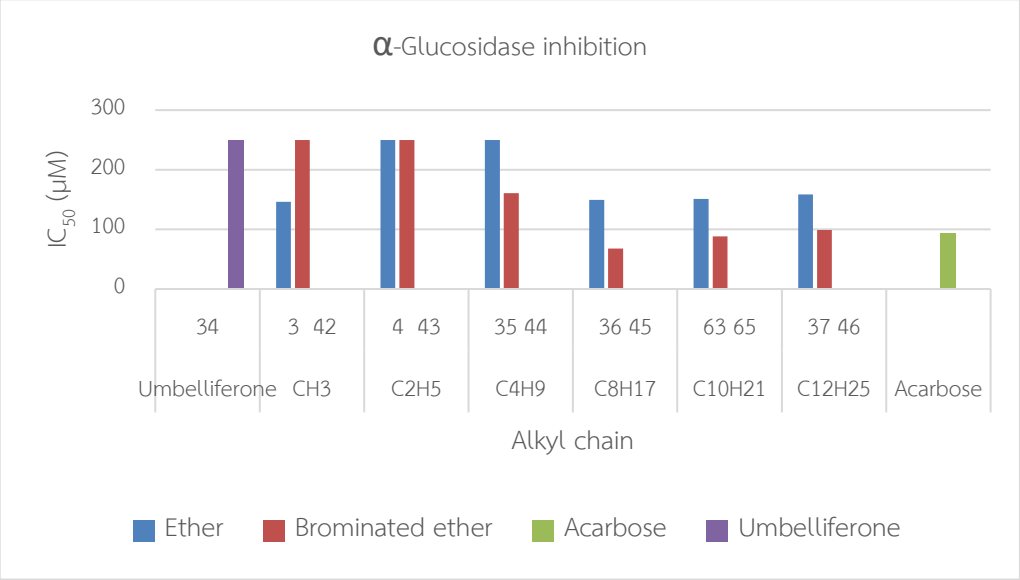
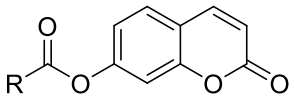


Figure 3.6 **α**-Glucosidase inhibition of ether, brominated ether derivatives of umbelliferone and acarbose.

3.3.3 Ester derivatives of umbelliferone

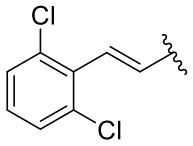
Ether and brominated ether derivatives of umbelliferone indicated the role of hydrophobic interaction to **α**-glucosidase inhibitory activity. Umbelliferone bearing ester substituent may also show this effect. Therefore, ester derivatives **47-53** were tested against **α**-glucosidase. Two new ester derivatives **54** and **55** were also tested. The results are presented in **Table 3.4**.

Table 3.4 Inhibition data of ester derivatives **48-55** and acarbose against **α**-glucosidase.

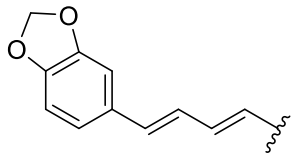


47: **R** = CH_3
48: R = C_3H_7
49: R = C_5H_{11}
50: R = C_7H_{15}
51: R = C_9H_{19}
52: R = $C_{11}H_{23}$
53: R = $C_{15}H_{31}$

54: R =



55: R =



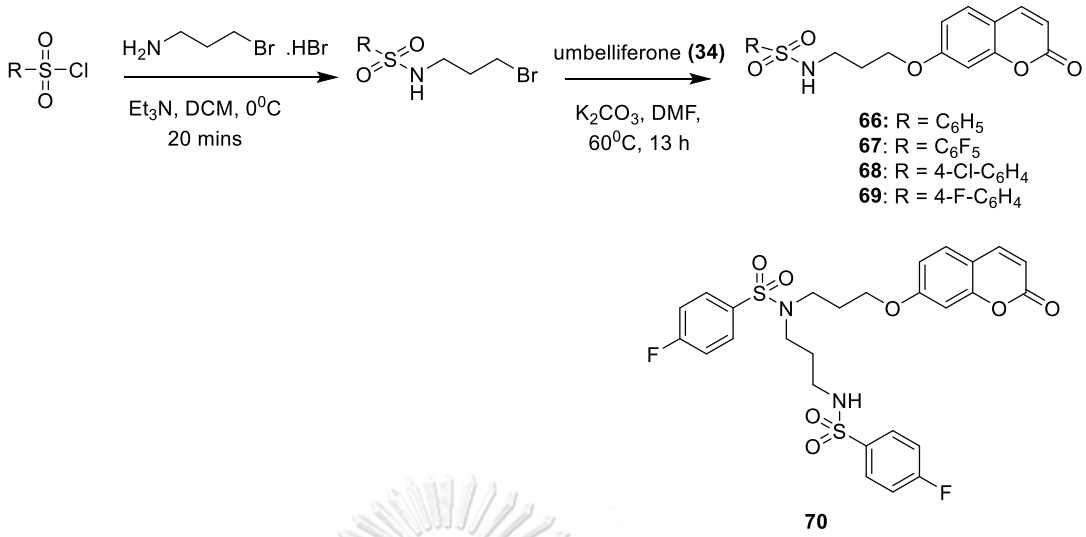
Entry	Compounds	IC_{50} (μM)
1	**47**	>200
2	**48**	>200
3	**49**	>200
4	**50**	>200
5	**51**	123.88 ± 0.97
6	**52**	137.76 ± 1.3
7	**53**	195.11 ± 1.31
8	**54**	>200
9	**55**	>200
10	Acarbose	93.63 ± 0.49

Although ester derivatives **47-50** inhibited the enzyme with IC_{50} > 200 μM, **51-53** illustrated some point of view of structure-activity relationship. The ether derivatives **37** and **63** possessing ten and twelve carbon atoms in side chain showed inhibitory activity at 151.26±0.49 and 158.4±0.90 μM, respectively whereas **51**, **52** containing the same number of carbon atoms increased the activity to 123.88±0.97 and

137.76±1.3 μM, correspondingly. It seems like ester derivatives of umbelliferone showed better activities than corresponding ether derivatives. On the other hand, ester derivatives of umbelliferone represented the increasing of the hydrophobicity of compound resulted in increasing the activity until ten carbon atoms (entry 5). However, the esters **52**, **53** possessing twelve and sixteen carbon atoms in side chain revealing less inhibitory activity (entries 6, 7), indicated that the compound bearing too hydrophobic part did not display good anti-**α**-glucosidase activity. Therefore **α**-glucosidase activity was affected by hydrophobic effect that was proved by number of carbon atoms in side chain of not only ethers, brominated ethers but also ester derivatives of umbelliferone. In addition, two new ester derivatives of umbelliferone **54** and **55** were not good inhibitors (IC_{50} > 200 μM).

3.3.4 Sulfonamide umbelliferone derivatives

Sulfonamide coumarins have been reported as effective **α**-glucosidase inhibitors.[62] These results encouraged to synthesize sulfonamide coumarins (**Scheme 3.2**) and evaluate for their **α**-glucosidase inhibitory activity. All new compounds **66-70** were characterized by ^{1}H and ^{13}C NMR (**Tables 3.5-3.8**) and confirmed structures by high resolution mass spectra (HRMS).



Scheme 3.2 Synthesis of sulfonamide umbelliferone derivatives **66**-**70**

Table 3.5 ^{1}H NMR chemical shift assignment of **66-69**

Position	δ_H (ppm)			
	66	**67**	**68**	**69**
3	6.27 (d, 1H, *J* = 9.2 Hz)	6.36 (d, 1H, *J* = 9.6 Hz)	6.21 (d, 1H, *J* = 9.6 Hz)	6.21 (d, 1H, *J* = 9.2 Hz)
4	7.96 (d, 1H, *J* = 9.6 Hz)	7.99 (d, 1H, *J* = 9.6 Hz)	7.89 (d, 1H, *J* = 9.6 Hz)	7.94-7.88 (m, 1H)
5	7.80-7.78 (m, 1H)	7.77 (d, 1H, *J* = 8.8 Hz)	7.56-7.54 (m, 1H)	7.56 (d, 1H, *J* = 8.8 Hz)
6	7.71 (br, 1H)	7.25 (d, 1H, *J* = 8.4 Hz)	6.84 (dd, 1H, *J* = 8.8, 2.4 Hz)	6.86 (dd, 1H, *J* = 8.8, 2.4 Hz)
8	6.88-6.86 (m, 1H)	7.14 (s, 1H)	6.79 (s,1H)	6.81 (s, 1H)
7-$OC_3H_7NHSO_2R$	C_6H_5	C_6F_5	4-Cl-C_6H_4	4-F-C_6H_4
	4.04 (t, 2H, *J* = 5.6 Hz, H1′), 2.93 (br, 2H, H2′), 1.84 (t, 2H, *J* = 6.4 Hz, H3′), 7.61-7.55 (m, 4H, H2-Ar, H6-Ar, H3-Ar, H5-Ar), 7.80-7.78 (m, 1H, H4-Ar)	4.04 (t, 4H, *J* = 7.6 Hz, H1′, H3′), 2.24 (quint, 2H, *J* = 7.6 Hz, H2′)	4.11 (t, 2H, *J* = 6.0 Hz, H1′), 2.01-1.96 (m, 2H, H2′), 3.18 (t, 2H, *J* = 6.0 Hz, H3′), 7.85 (d, 2H, *J* = 8.4 Hz, H2-Ar, H6-Ar), 7.56-7.54 (m, 2H, H3-Ar, H5-Ar)	4.14 (t, 2H, *J* = 6.0 Hz, H1′), 2.02-1.97 (m, 2H, H2′), 3.16 (q, 2H, *J* = 6.0 Hz, H3′), 7.94-7.88 (m, 2H, H2-Ar, H6-Ar), 7.31 (m, 2H, H3-Ar, H5-Ar)

Table 3.6 ^{13}C NMR chemical shift assignment of **66-69**

Position	δ_C (ppm)			
	66	**67**	**68**	**69**
2	160.3	159.9	161.1	164.4
3	101.1	104.4	102.0	102.1
4	144.3	144.1	144.6	144.6
5	129.4	131.1	129.5	130.1
6	112.7	113.6	113.6	113.7
7	161.6	160.3	163.0	166.9
8	112.4	116.0	113.5	113.5
9	155.3	156.5	156.8	156.9
10	112.3	116.7	113.5	113.6
7-$OC_3H_7NHSO_2R$	C_6H_5	C_6F_5	4-Cl-C_6H_4	4-F-C_6H_4
	65.4 (C1′ and C3′), 28.6 (C2′), 140.3 (C1-Ar), 129.2 (C2-Ar and C6-Ar), 126.4 (C3-Ar and C5-Ar), 132.3 (C4-Ar)	52.2 (C1′ and C3′), 15.9 (C2′), 147.7 (C1-Ar), 144.3 (C2-Ar and C6-Ar), 141.6 (C3-Ar and C5-Ar), 145.1 (C4-Ar)	66.3 (C1′ and C3′), 40.6 (C2′), 140.8 (C1-Ar), 130.1 (C2-Ar and C6-Ar), 129.5 (C3-Ar and C5-Ar), 138.8 (C4-Ar)	66.4 (C1′ and C3′), 40.6 (C2′), 163.0 (C1-Ar), 130.7 (C2-Ar), 130.6 C6-Ar), 117.0 (C3-Ar), 116.8 C5-Ar), 161.0 (C4-Ar)

Table 3.7 ^{1}H NMR chemical shift assignment of **70**

Position	δ_H (ppm)
	70
3	6.26 (d, 1H, *J* = 9.6 Hz)
4	7.63 (d, 1H, *J* = 9.6 Hz)
5	7.36 (d, 1H, *J* = 8.8 Hz)
6	6.78 (dd, 1H, *J* = 8.8, 2.0 Hz)
8	6.71 (s, 1H)
7-$OC_3H_7N(SO_2R)$-$C_3H_7NHSO_2R$	4-F-C_6H_4
	3.97 (t, 2H, *J* = 5.6 Hz, H1′), 2.02 (t, 2H, *J* = 5.6 Hz, H2′), 3.04 (br, 2H, H3′), 3.28 (t, 2H, *J* = 6.8 Hz, H1′′), 1.81 (t, 2H, *J* = 5.6 Hz, H2′′), 3.25 (t, 2H, *J* = 6.4 Hz, H3′′), 7.87 (dd, 2H, *J* = 8.4, 4.8 Hz, H2′-Ar, H6′-Ar), 7.17 (q, 4H, *J* = 8.4 Hz, H3′-Ar, H5′-Ar, H3′′-Ar, H5′′-Ar), 7.80 (dd, 2H, *J* = 8.8, 5.2 Hz, H2′′-Ar, H6′′-Ar)

Table 3.8 ^{13}C NMR chemical shift assignment of **70**

Position	δ_C (ppm)
	70
2	161.2
3	101.6
4	143.5
5	129.0
6	113.5
7	161.8
8	112.8
9	155.9
10	112.9
7-$OC_3H_7N(SO_2R)$-	4-F-C_6H_4
$C_3H_7NHSO_2R$	65.5 (C1′), 29.1 (C2′), 46.3 (C3′), 39.9 (C1′′), 28.7 (C2′′), 29.8 (C3′′), 166.6 (C1-Ar), 166.5 (C1-Ar), 164.0 (C4-Ar), 163.9 (C4-Ar), 130.0 (C2-Ar), 129.9 (C2-Ar), 129.9 (C6-Ar), 129.8 (C6-Ar), 116.8 (C3-Ar), 116.6 (C3-Ar), 116.6 (C5-Ar), 116.4 (C5-Ar)

Table 3.9 Inhibition data of sulfonamide umbelliferone derivatives **66-70** and acarbose against **α**-glucosidase.

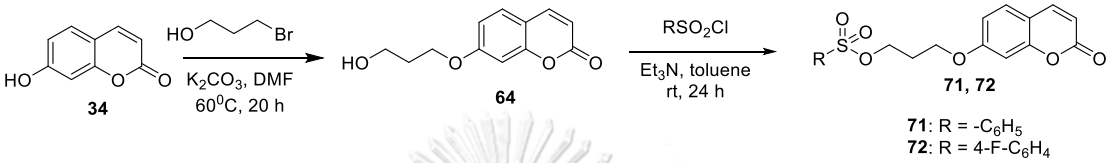
Entry	Compounds	IC_{50} (μM)
1	**66**	>200
2	**67**	>200
3	**68**	>200
4	**69**	140.6 ± 0.15
5	**70**	36.6 ± 0.86
6	Acarbose	93.63 ± 0.49

Among the synthesized sulfonamide coumarins **66-69**, only sulfonamide **69** containing -F at *para* position of aromatic ring showed the good activity with **α**-glucosidase (IC_{50} = 140.6±0.15 μM). Interestingly, when introducing more sulfonamide group in **70**, the activity increased significantly, which was three times better than a standard drug. Although sulfonamide coumarins was reported as good **α**-glucosidase inhibitors, their chemical structure contained one sulfonamide group in side chain at C-3.[62] In this study, **70** incorporating two sulfonamide groups in side chain at position 7 of umbelliferone was evaluated as a good inhibitor against **α**-glucosidase. This was the first discovery about structure-activity relationship, more sulfonamide substituent of umbelliferone derivative, the better activity towards anti-**α**-glucosidase was observed.

3.3.5 Sulfonate umbelliferone derivatives

To discover the role of sulfonamide of umbeliiferone derivatives, selected sulfonate umbelliferone derivatives were synthesized (**Scheme 3.3**). Ether derivative

of umbelliferone **64** was synthesized as an intermediate compound (**Scheme 3.3**). This derivative was characterized by ^{1}H and ^{13}C NMR. The spectra assignment of **64** are assembled in **Table 3.10**. **64** was tested for the inhibitory activity, unfortunately this compound displayed not good activity (IC_{50} > 200 μM).



Scheme 3.3 Synthesis of sulfonate umbelliferone derivatives **71**, **72**

Table 3.10 ^{13}C NMR chemical shift assignment of ether derivative **64**

Position	**64**	
	δ_H (ppm)	δ_C (ppm)
2		161.5
3	6.20 (d, 1H, *J* = 9.2 Hz)	113.0
4	7.60 (d, 1H, *J* = 9.6 Hz)	143.6
5	7.33 (d, 1H, *J* = 8.4 Hz)	128.9
6	6.81 (dd, 1H, *J* = 8.4, 2.4 Hz)	113.0
7		162.3
8	6.77 (d, 1H, *J* = 2.0 Hz)	101.5
9		155.9
10		112.7
HO	4.14 (t, 2H, *J* = 6.4 Hz, H1′), 2.05 (m, 2H, H2′), 3.84 (t, 2H, *J* = 6.0 Hz, H3′)	65.8 (C1′), 31.9 (C2′), 59.5 (C3′)

Two selected sulfonate coumarins **71**, **72** were synthesized from the reaction of ether **64** and aryl sulfonyl chloride. Their ^{1}H and ^{13}C NMR characterization are represented in **Tables 3.11** and **3.12**. The new derivatives were confirmed their structures by HRMS.

Table 3.11 ^{1}H NMR chemical shift assignment of **71** and **72.**

Position	δ_H (ppm)	
	71	**72**
3	6.24 (d, 1H, *J* = 9.2 Hz)	6.22 (d, 1H, *J* = 9.2 Hz)
4	7.63 (d, 1H, *J* = 9.6 Hz)	7.89 (d, 1H, *J* = 9.6 Hz)
5	7.34 (d, 1H, *J* = 8.8 Hz)	7.55 (d, 1H, *J* = 8.8 Hz)
6	6.72 (d, 1H, *J* = 8.8 Hz)	6.81 (dd, 1H, *J* = 8.4, 2.4 Hz)
8	6.66 (s, 1H)	6.77 (d, 1H, *J* = 2.4 Hz)
7-	C_6H_5	4-F-C_6H_4
$OC_3H_7OSO_2R$	4.26 (t, 2H, *J* = 6.0 Hz, H1′), 2.16 (quint, 2H, *J* = 5.6 Hz, H2′), 4.02 (t, 2H, *J* = 5.6 Hz, H3′), 7.88 (d, 2H, *J* = 8.0 Hz, H2-Ar and H6-Ar), 7.48 (t, 2H, *J* = 7.2 Hz, H3-Ar and H5-Ar), 7.58 (t, 1H, *J* = 7.2 Hz, H4-Ar)	4.32 (t, 2H, *J* = 6.0 Hz, H1′), 2.18 (quint, 2H, *J* = 6.0 Hz, H2′), 4.12 (t, 2H, *J* = 6.0 Hz, H3′), 7.99 (m, 2H, H2-Ar, H6-Ar), 7.33 (t, 2H, *J* = 8.8 Hz, H3-Ar, H5-Ar)

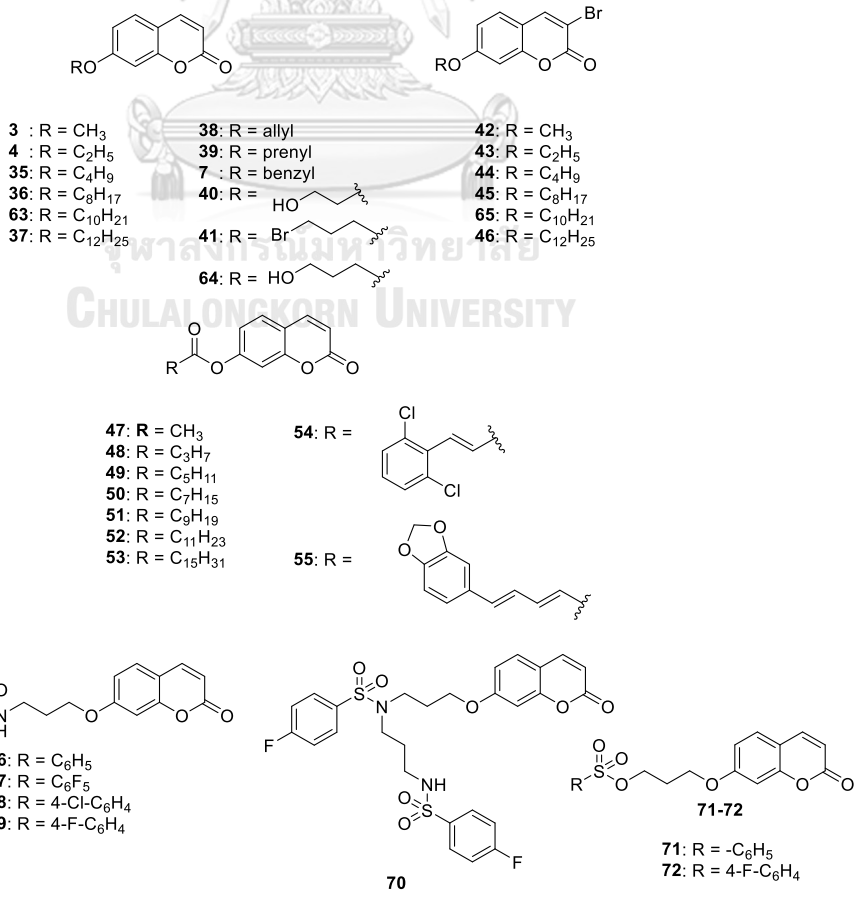
Table 3.12 ^{1}C NMR chemical shift assignment of **71** and **72.**

Position	δ_C (ppm)	
	71	**72**
2	161.2	165.3
3	101.7	102.1
4	143.4	144.5
5	129.4	130.1
6	113.4	113.8
7	161.7	167.8
8	112.6	113.3
9	155.9	156.8
10	112.9	113.7
7-$OC_3H_7OSO_2R$	C_6H_5	4-F-C_6H_4
	67.0 (C1′), 28.6 (C2′), 63.9 (C3′), 136.0 (C1-Ar), 128.9 (C2-Ar and C6-Ar), 127.9 (C3-Ar and C5-Ar), 133.9 (C4-Ar)	68.4 (C1′ and C3′), 64.8 (C2′), 162.7 (C1-Ar), 131.8 (C2-Ar), 131.7 (C6-Ar), 117.6 (C3-Ar), 117.4 C5-Ar), 160.9 (C4-Ar)

The inhibition data of two sulfonate umbelliferone derivatives showed not good results (IC_{50} > 200 μM). Thereby, sulfonamide moiety demonstrated better activity against **α**-glucosidase than sulfonate substituent.

3.4 Conclusion

From umbelliferone (**34**), thirty-four derivatives including ether, brominated ether, ester, sulfonamide and sulfonate coumarins were synthesized and elucidated their structures by appropriate spectroscopic techniques. Among them, thirteen compounds were reported for the first time. These substrates were characterized by using ^{1}H, ^{13}C and HR-ESI-MS, and the summary of their structures are displayed below:



To evaluate the potential of coumarins against **α**-glucosidase, all derivatives were tested. Among them, three compounds **45**, **65**, **70** demonstrated excellent inhibitory activity even better than a positive control–acarbose. Moreover, the **α**-glucosidase was affected by hydrophobic effect that was proved by number of carbon atoms in side chain of ether, brominated ether and ester derivatives of umbelliferone. Furthermore, sulfonamide substituent illustrated effectivity against the enzyme.

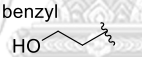
CHAPTER 4

CONCLUSION

In this study, umbelliferone derivatives were synthesized and tested with two enzymes, carbonic anhydrase II and α-glucosidase. Unfortunately, carbonic anhydrase II inhibitory activity of some umbelliferone derivatives was unrepeatable as expectation. Nonetheless, certain derivatives exhibited promising anti α-glucosidase inhibitors. Thirty-four derivatives of umbelliferone were synthesized and evaluated for the inhibitory activity. Structures of all synthesized derivatives are listed below:

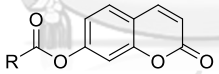
3 : R = CH_3
4 : R = C_2H_5
35: R = C_4H_9
36: R = C_8H_{17}
63: R = $C_{10}H_{21}$
37: R = $C_{12}H_{25}$

38: R = allyl
39: R = prenyl
7 : R = benzyl
40: R =



41: R =

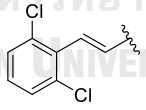
64: R =



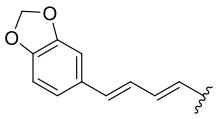
42: R = CH_3
43: R = C_2H_5
44: R = C_4H_9
45: R = C_8H_{17}
65: R = $C_{10}H_{21}$
46: R = $C_{12}H_{25}$

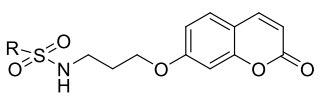
47: **R** = CH_3
48: R = C_3H_7
49: R = C_5H_{11}
50: R = C_7H_{15}
51: R = C_9H_{19}
52: R = $C_{11}H_{23}$
53: R = $C_{15}H_{31}$

54: R =

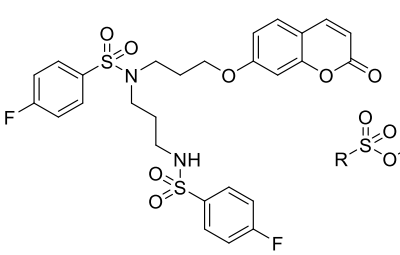


55: R =

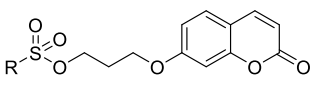




66: R = C_6H_5
67: R = C_6F_5
68: R = 4-Cl-C_6H_4
69: R = 4-F-C_6H_4



70



71-72

71: R = -C_6H_5
72: R = 4-F-C_6H_4

There are thirteen new compounds, among them, three new compounds **45**, **65**, **70** showed good inhibitory activities with IC_{50} of 68, 88, and 37 μM which were better than a positive control, acarbose (IC_{50} 94 μM). Moreover, hydrophobic effect was investigated to **α**-glucosidase inhibition for the first time. Besides that, bromo substituent at position 3 of brominated ether derivatives of umbelliferone as well as sulfonamide substituents could improve the inhibitory activity.

Suggestion for future work

Further study on the mechanism of the interaction between **45**, **65**, **70** and **α**-glucosidase should be considered. In addition, bromo substituent at position 3 of ether derivatives of umbelliferone improved significantly the inhibitory activity. Therefore, the effect of bromo substituent should be continuous studied. Furthermore, coumarins with a variety of sulfonamide substituents would be synthesized to evaluate for structure-activity relationship. This research proved that **α**-glucosidase was affected by hydrophobic effect of umbelliferone derivatives. This observation suggests further investigation on other types of coumarins.

APPENDIX

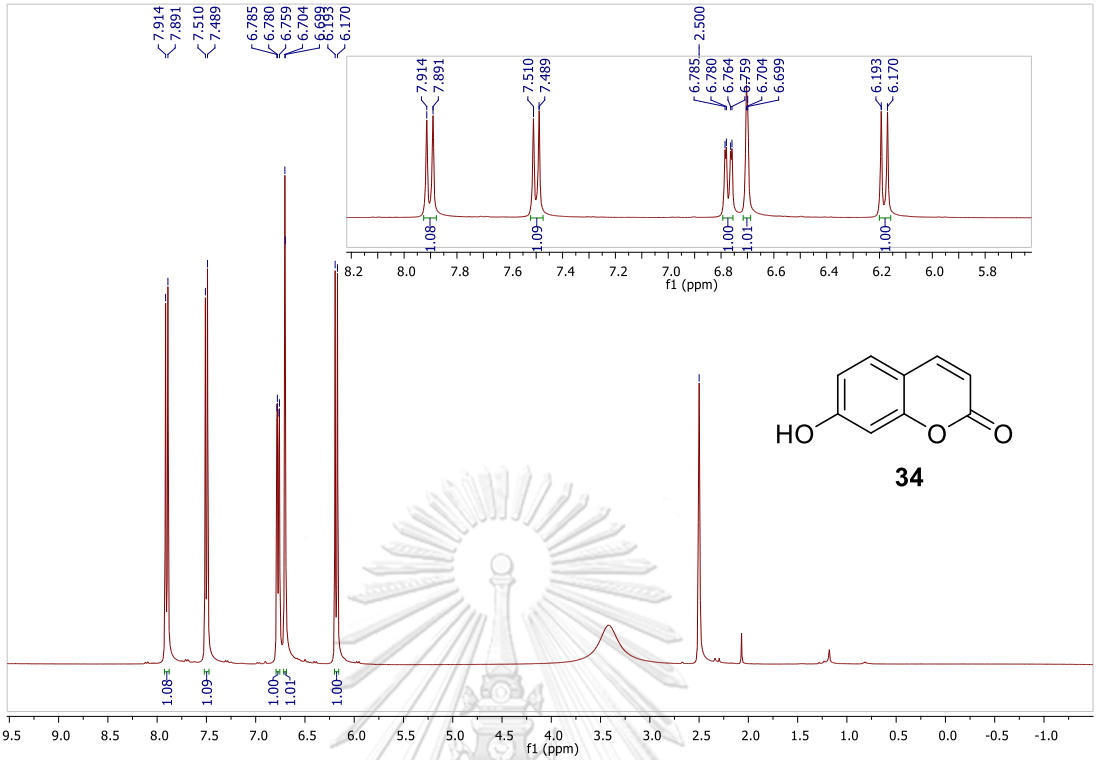


Figure A.1 ^{1}H-NMR (DMSO-d_6, 400 MHz) of umbelliferone (**34**).

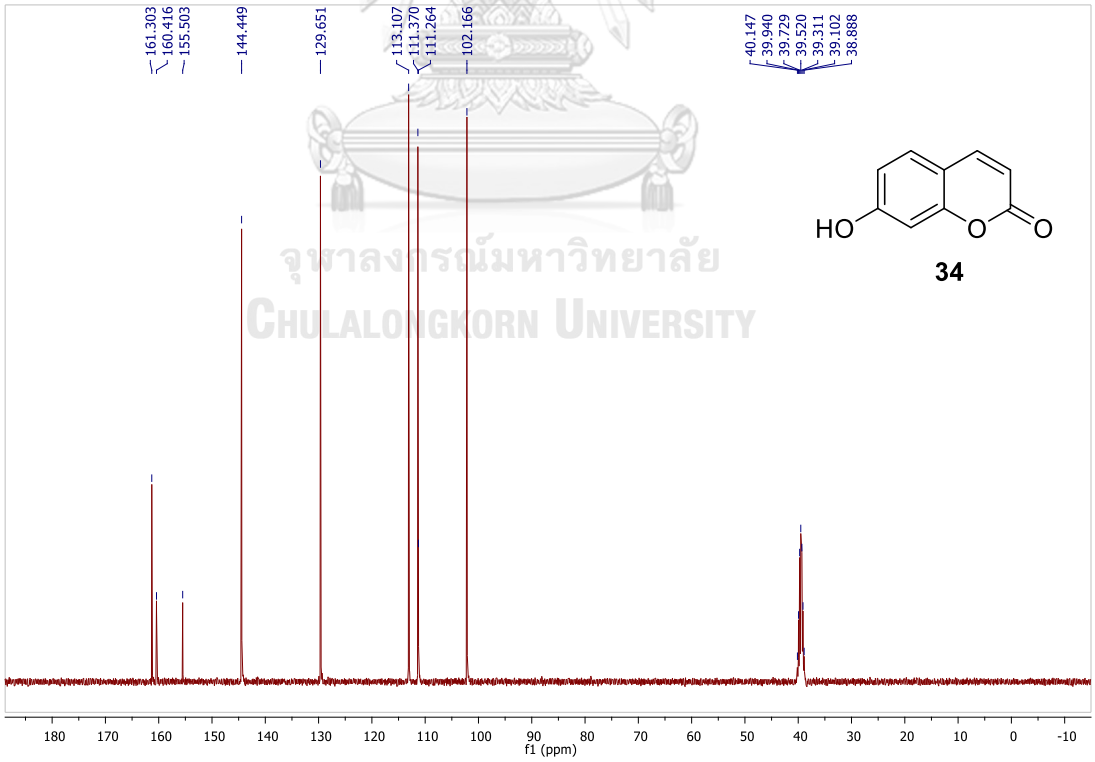


Figure A.2 ^{13}C-NMR (DMSO-d_6, 100 MHz) of umbelliferone (**34**).

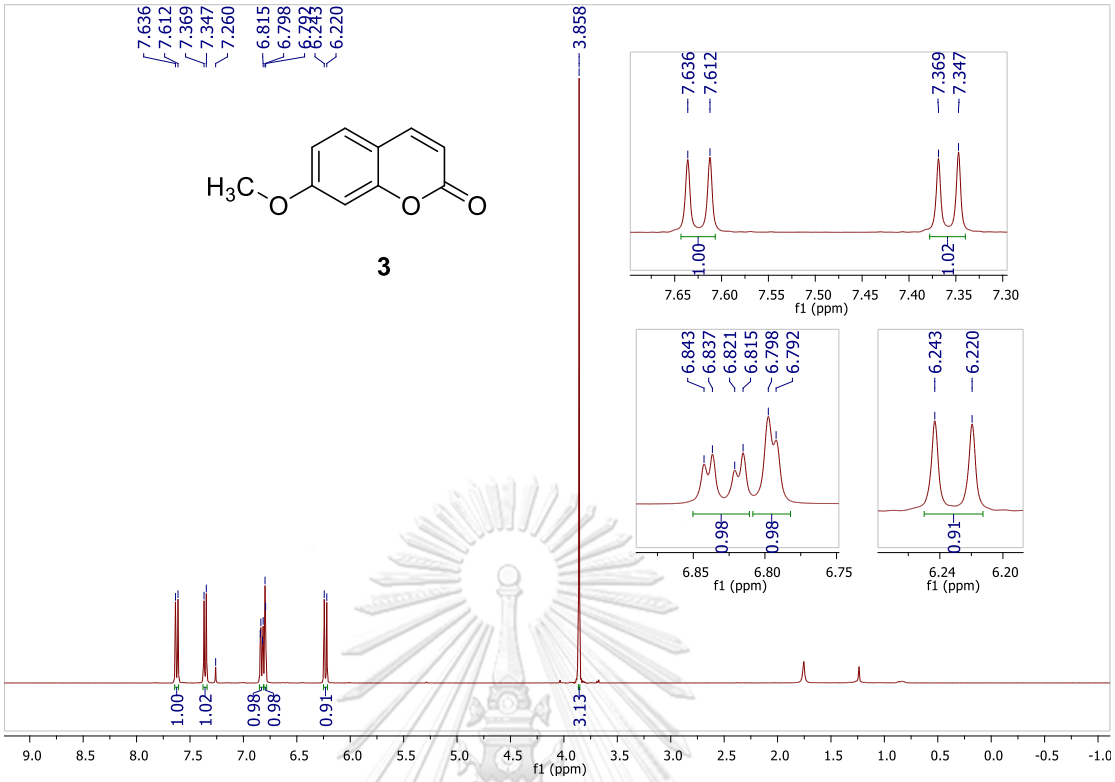


Figure A.3 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **3**.

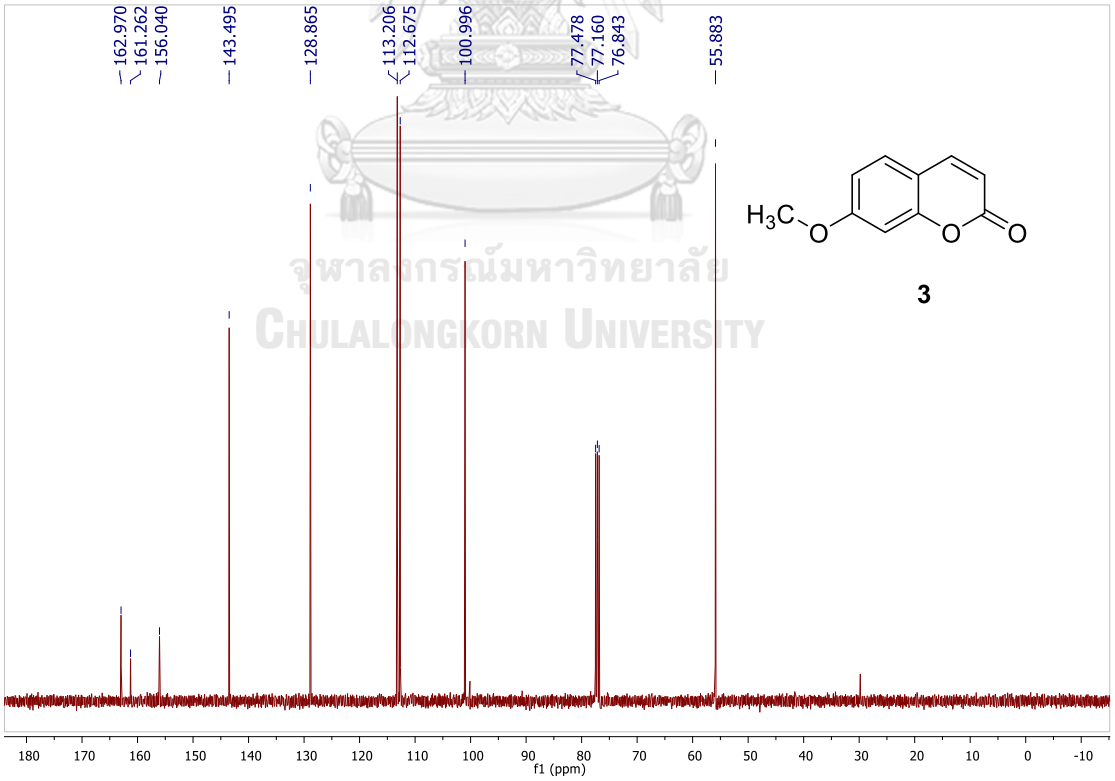


Figure A.4 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **3**.

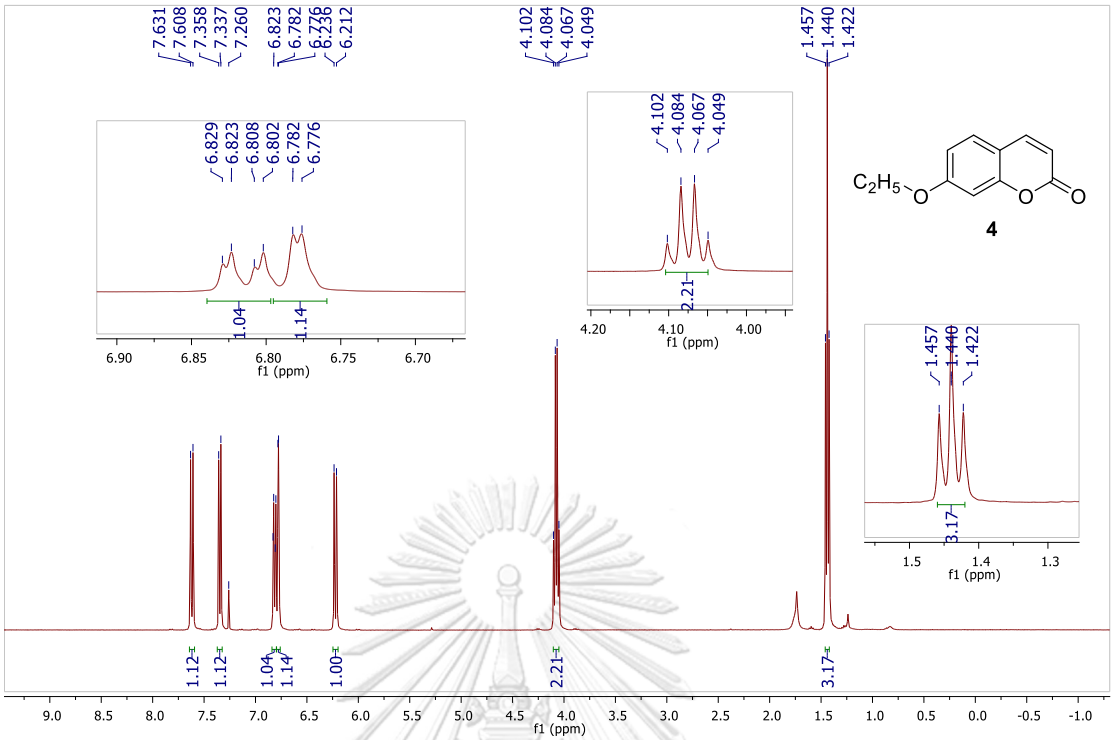


Figure A.5 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **4.**

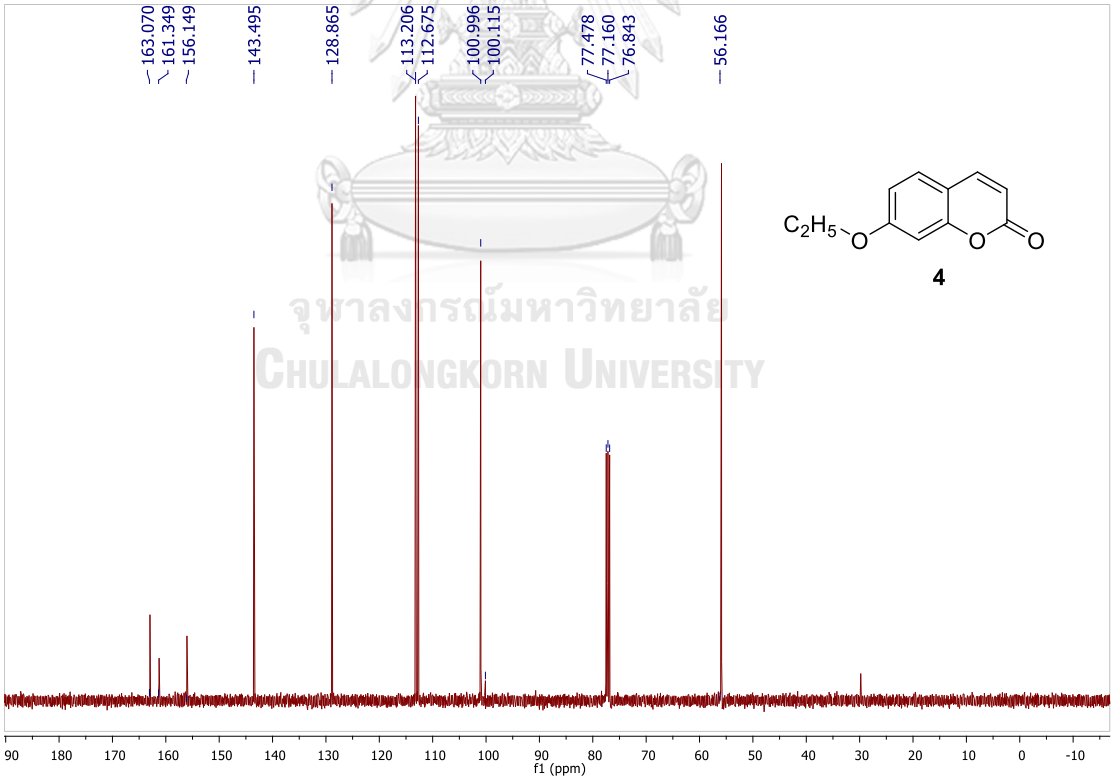


Figure A.6 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **4.**

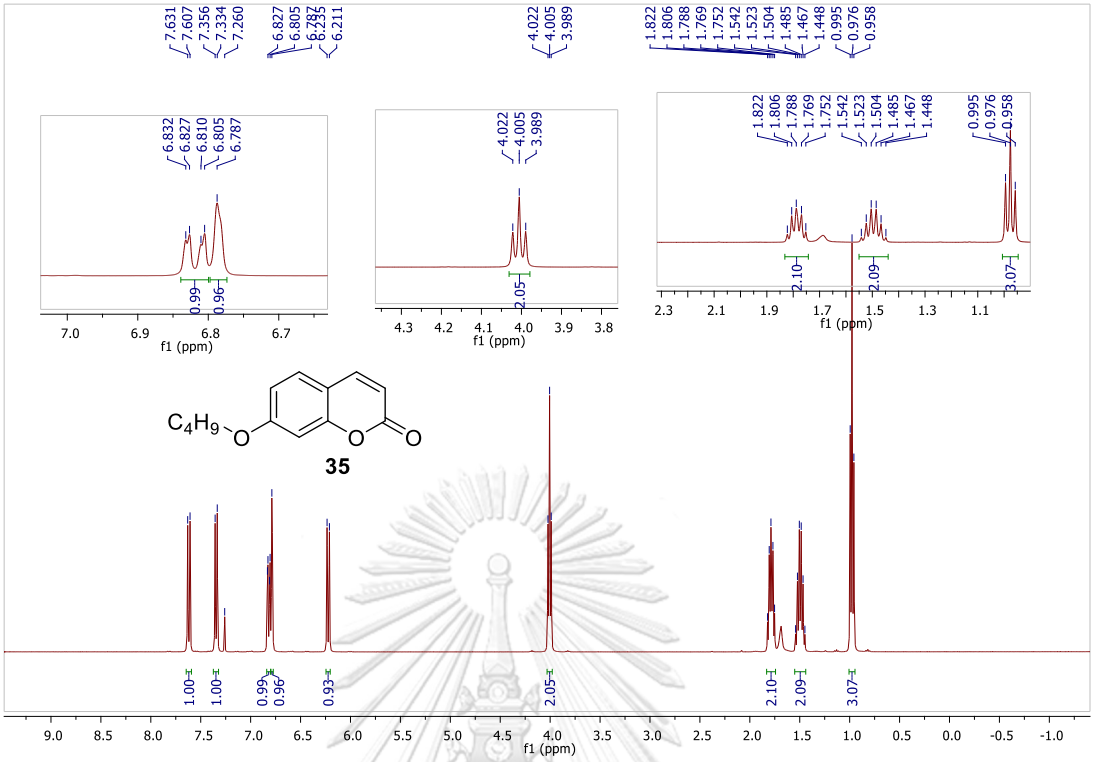


Figure A.7 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **35**.

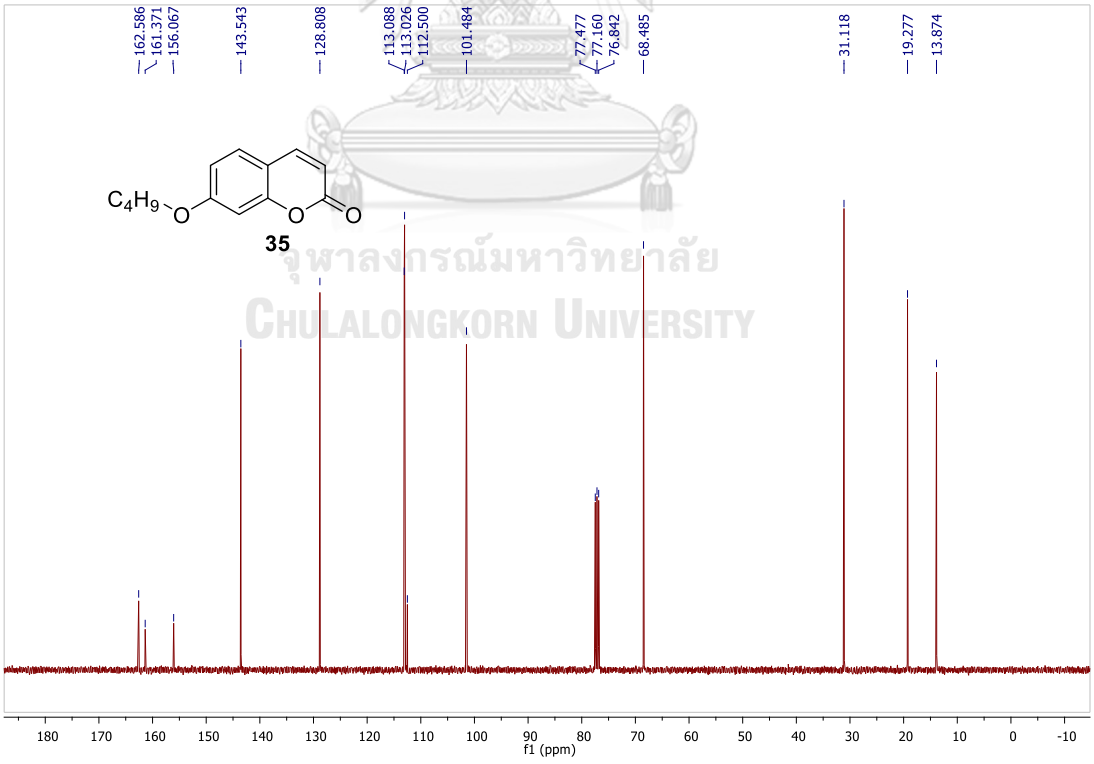


Figure A.8 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **35**.

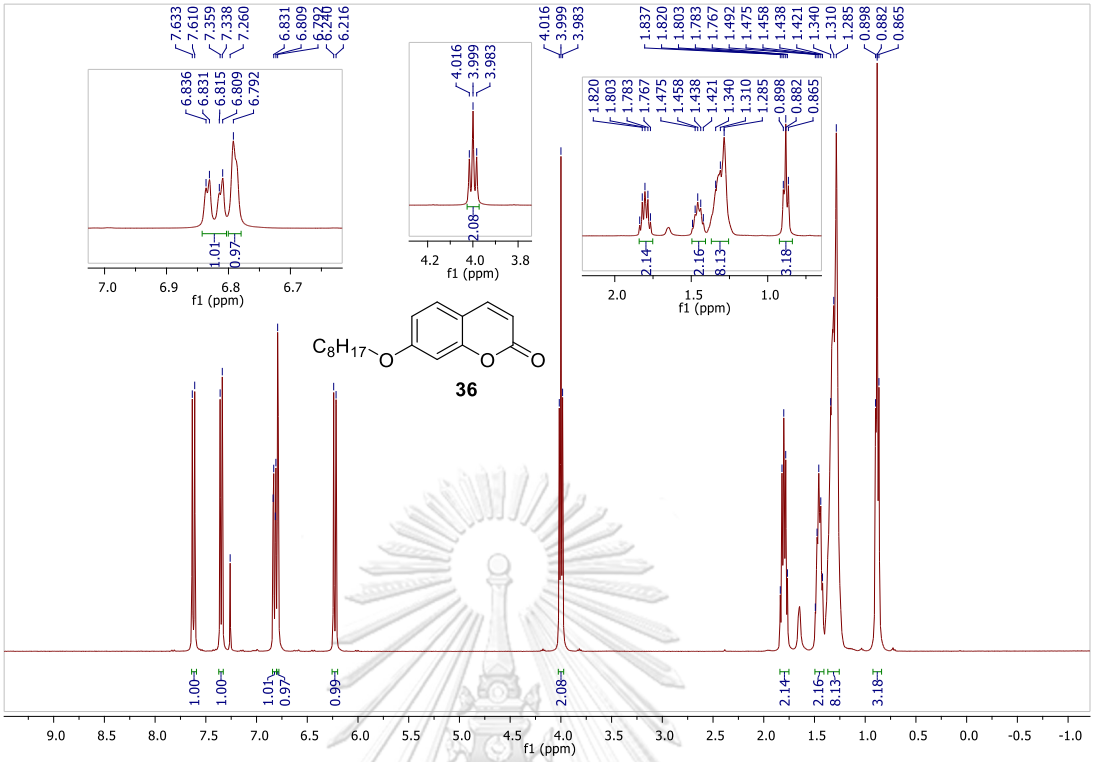


Figure A.9 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **36.**

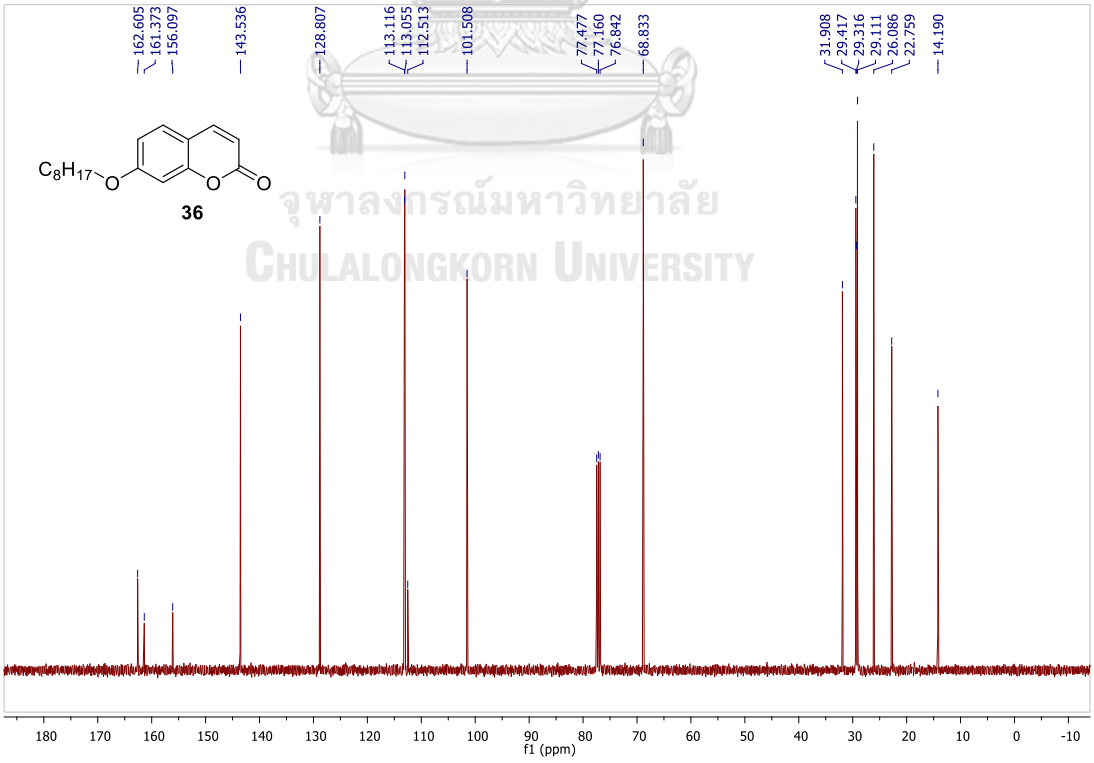


Figure A.10 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **36.**

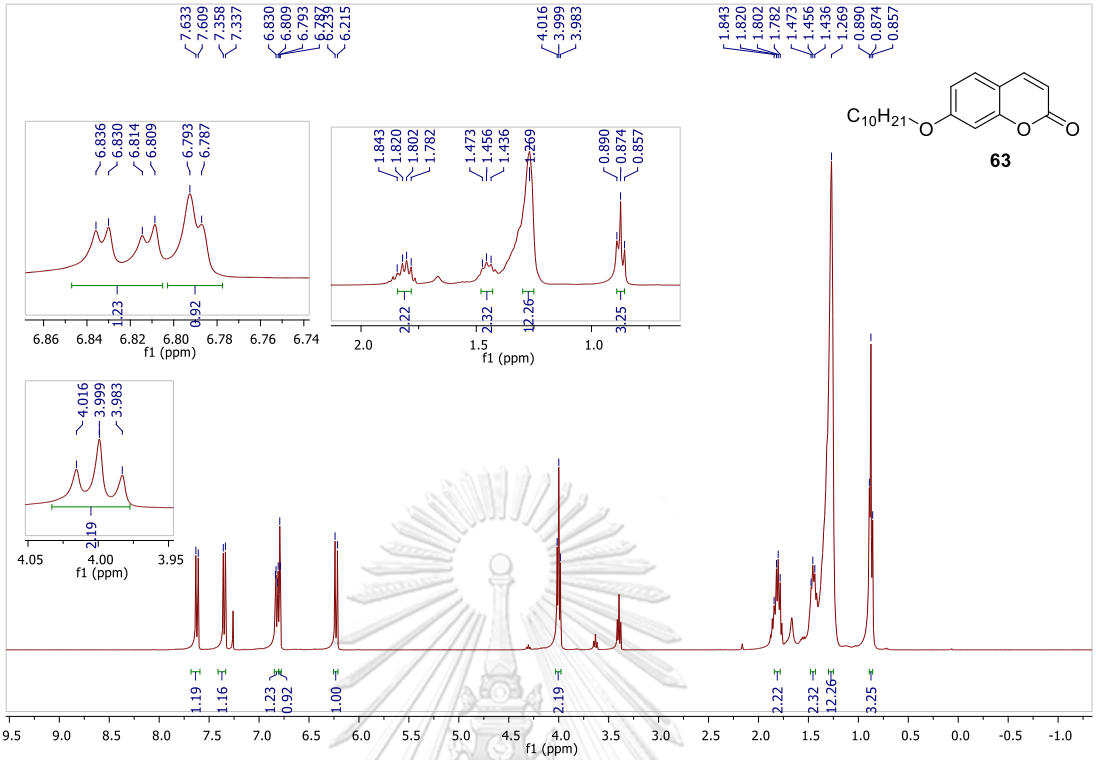


Figure A.11 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **63.**

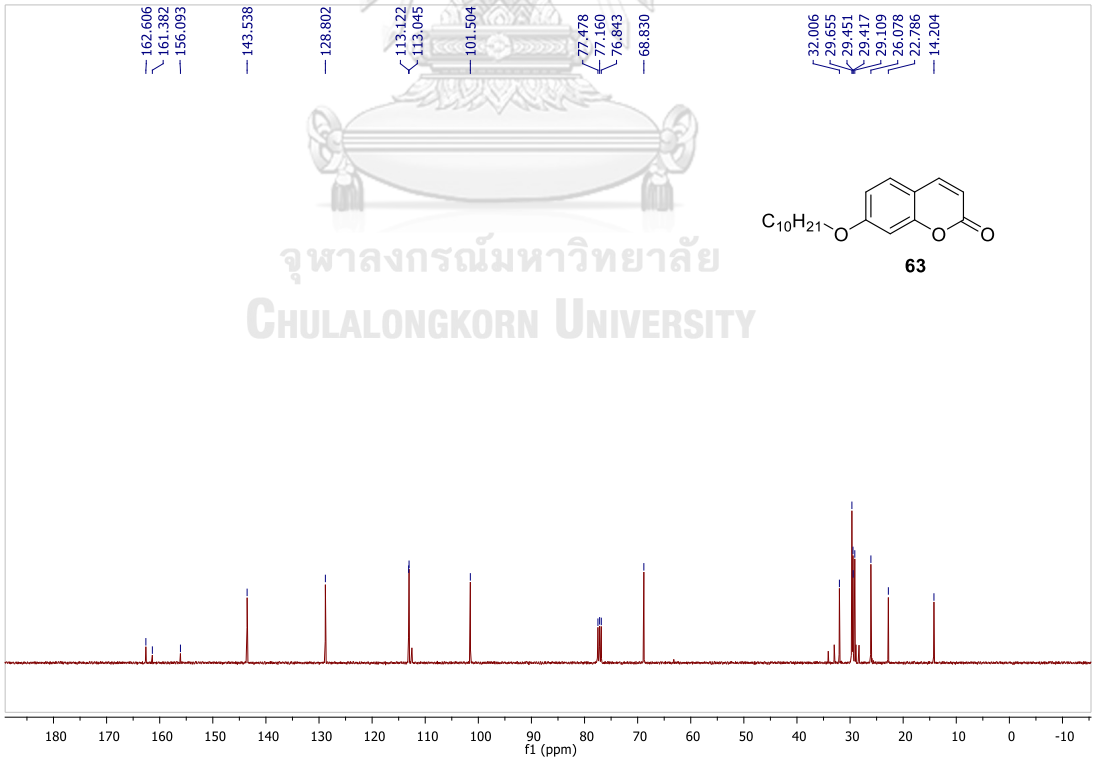


Figure A.12 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **63.**

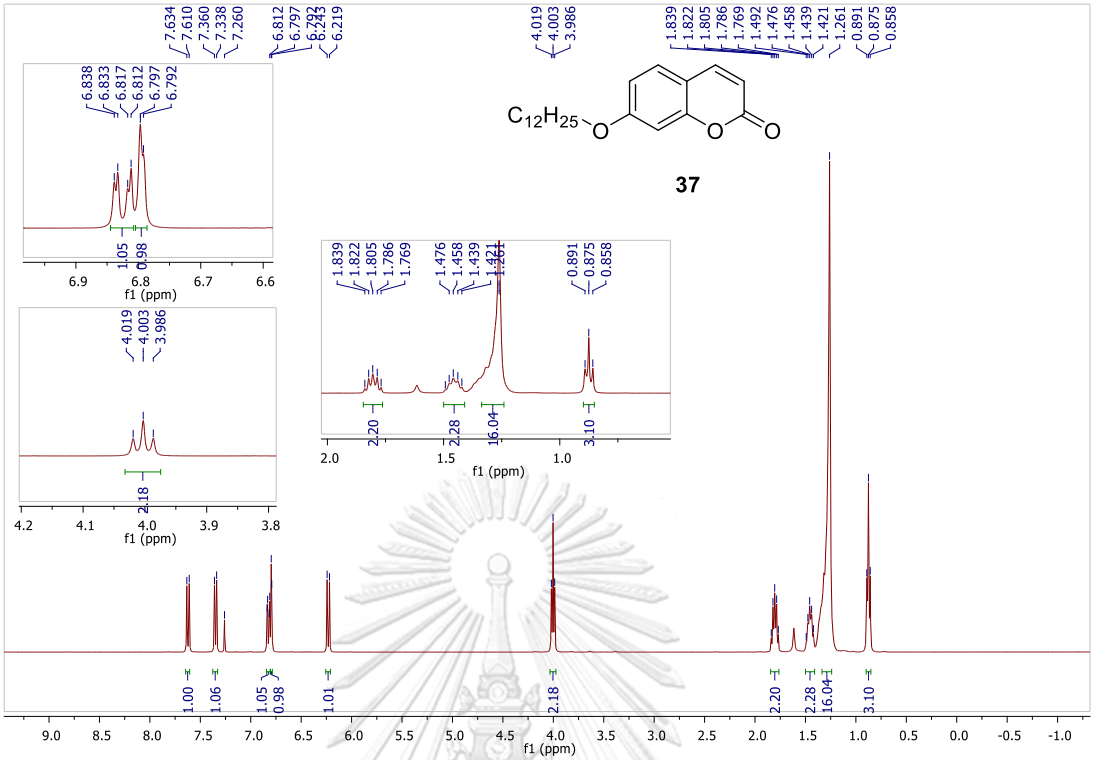


Figure A.13 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **37**.

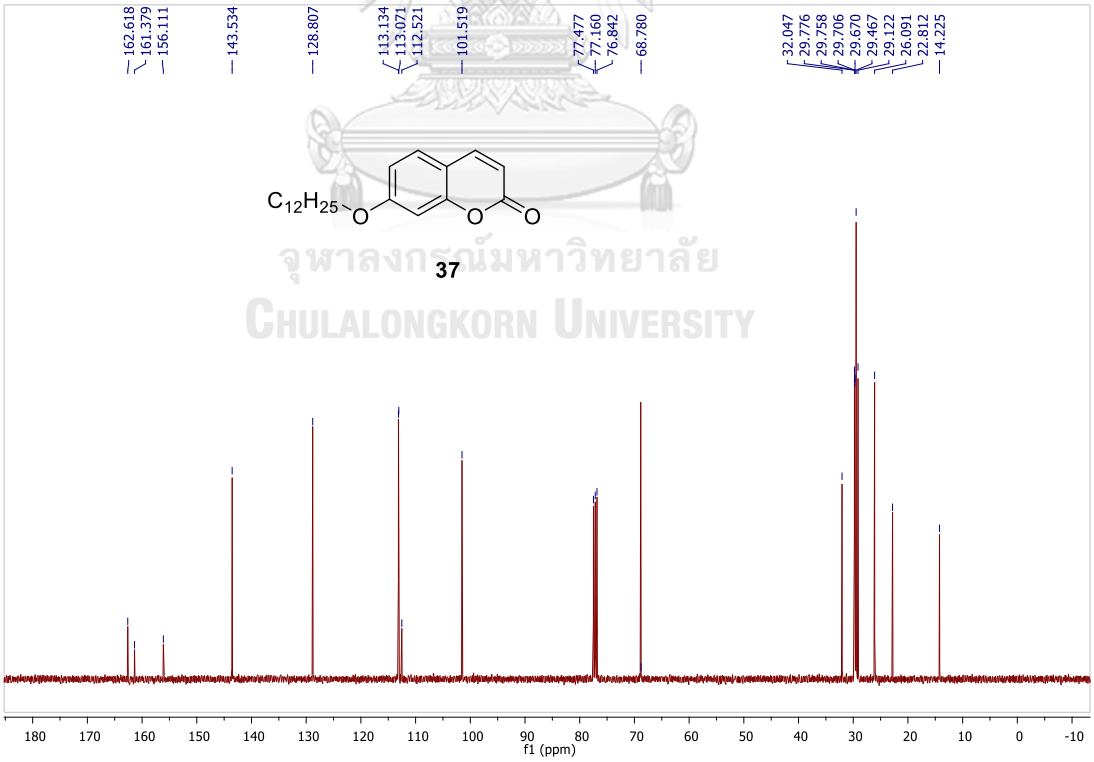


Figure A.14 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **37**.

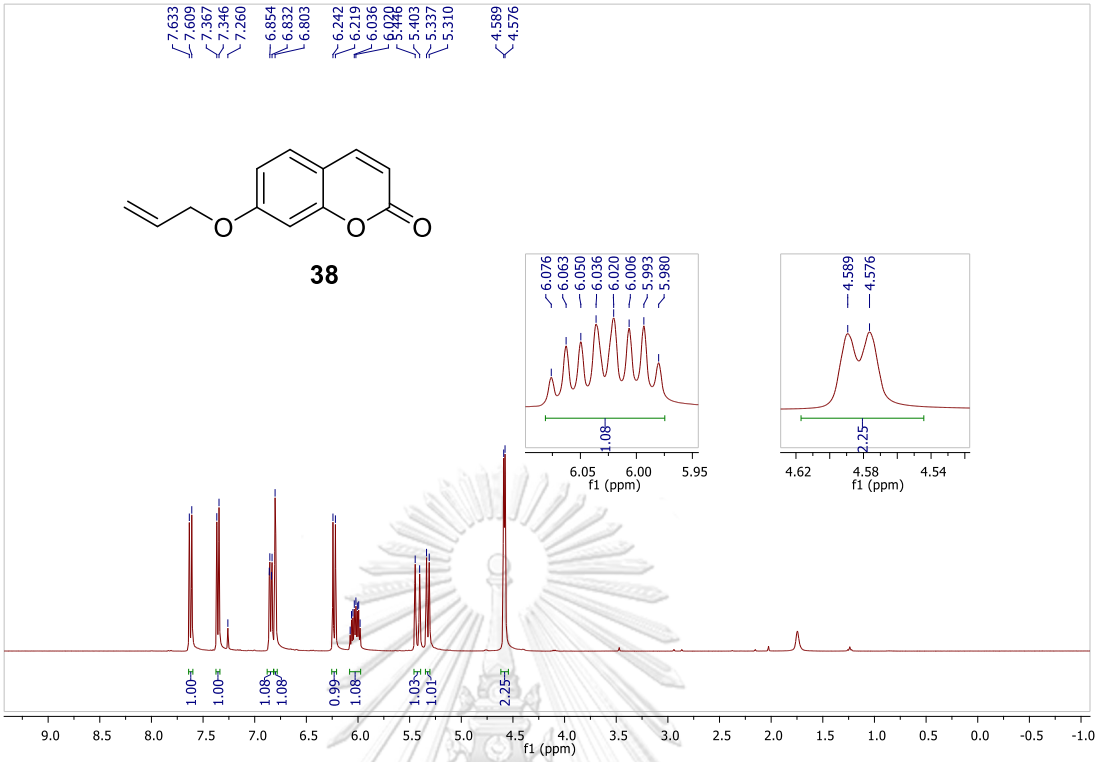


Figure A.15 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **38.**

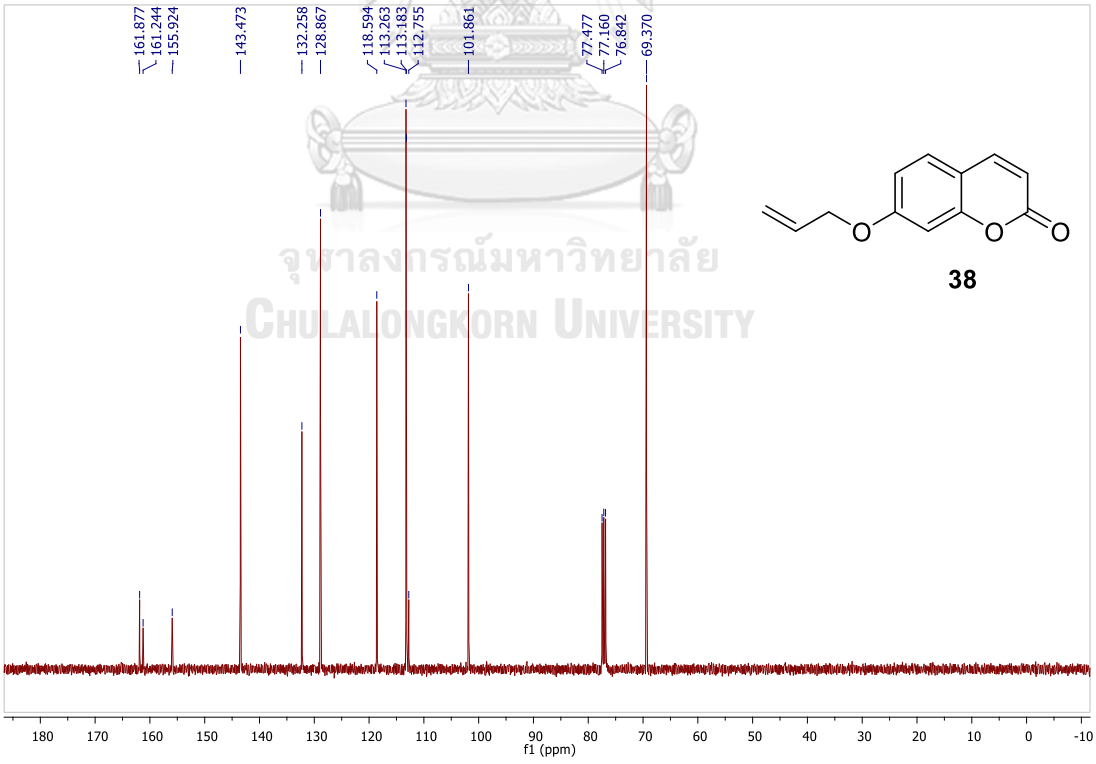


Figure A.16 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **38.**

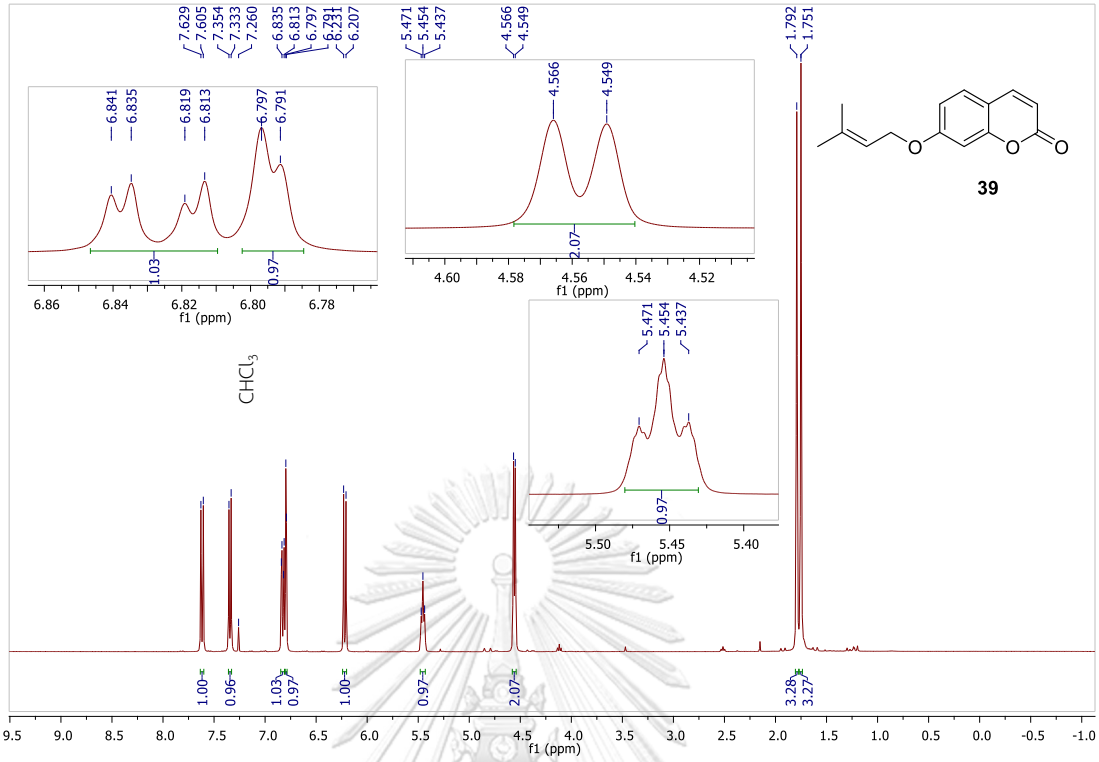


Figure A.17 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **39.**

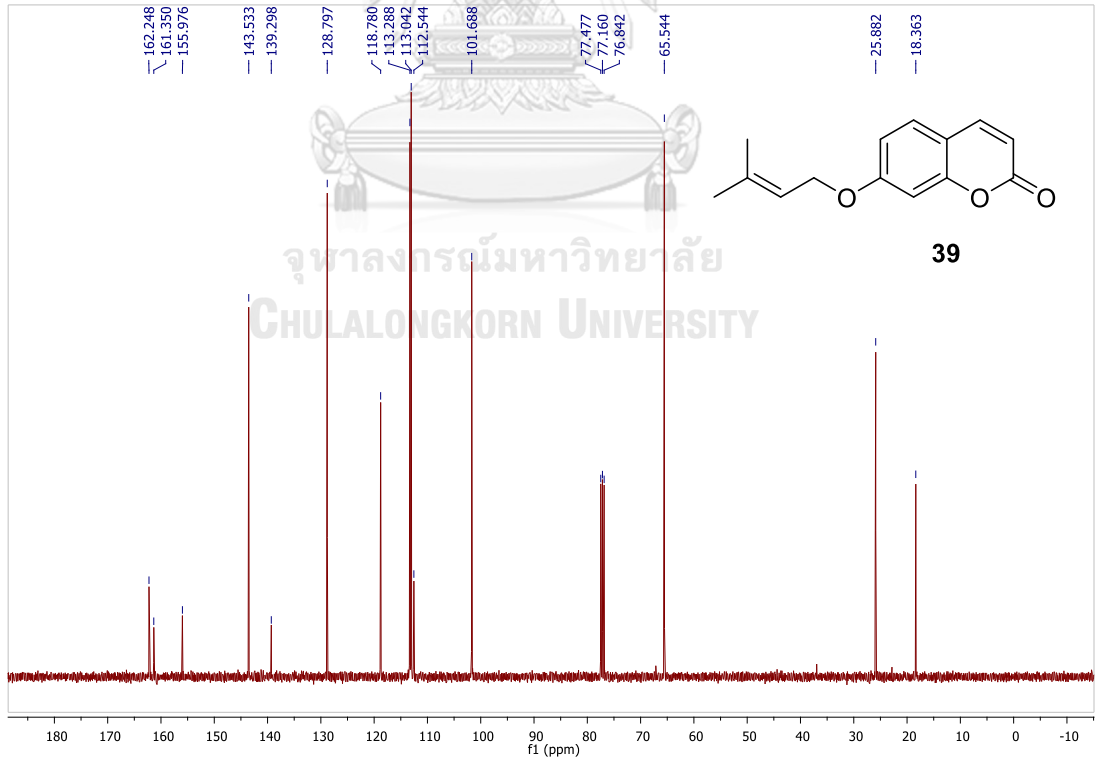


Figure A.18 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **39.**

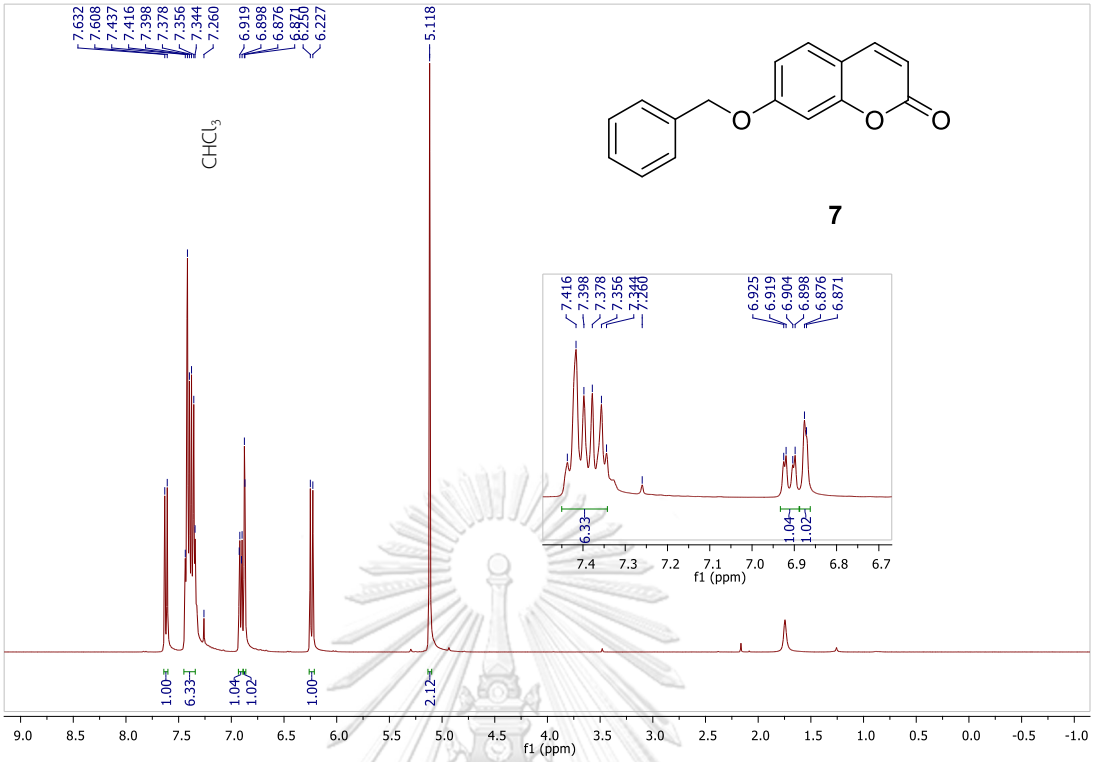


Figure A.19 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **7**.

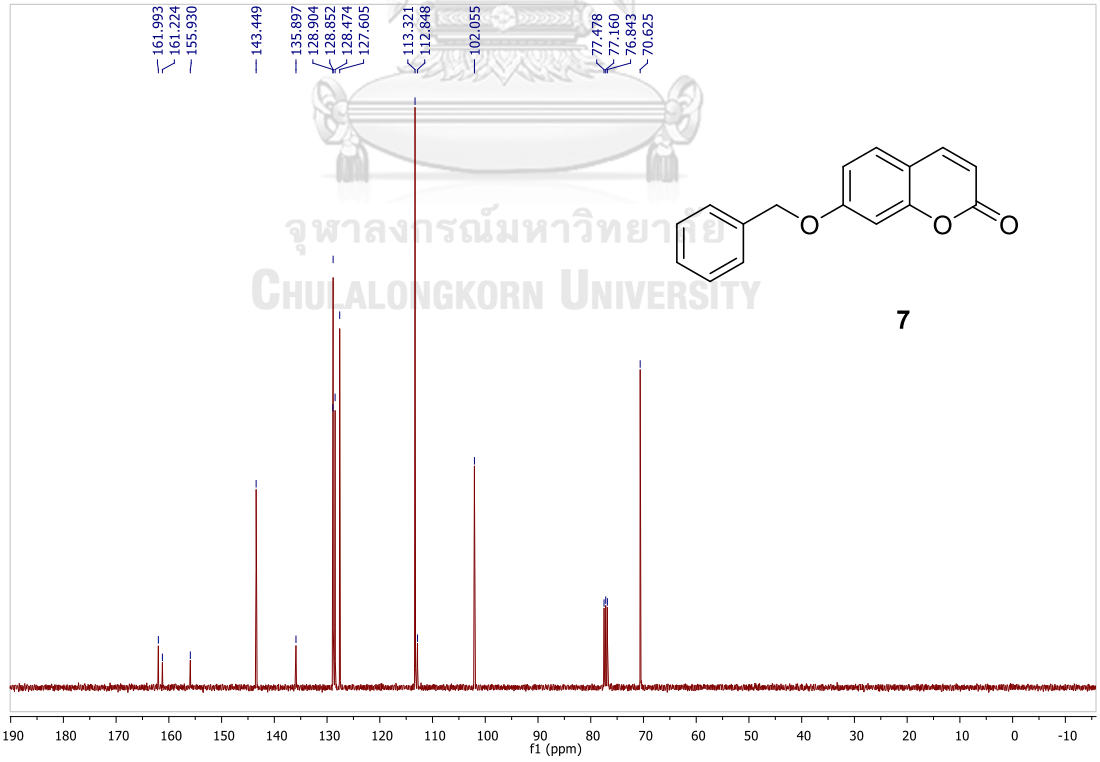


Figure A.20 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **7**.

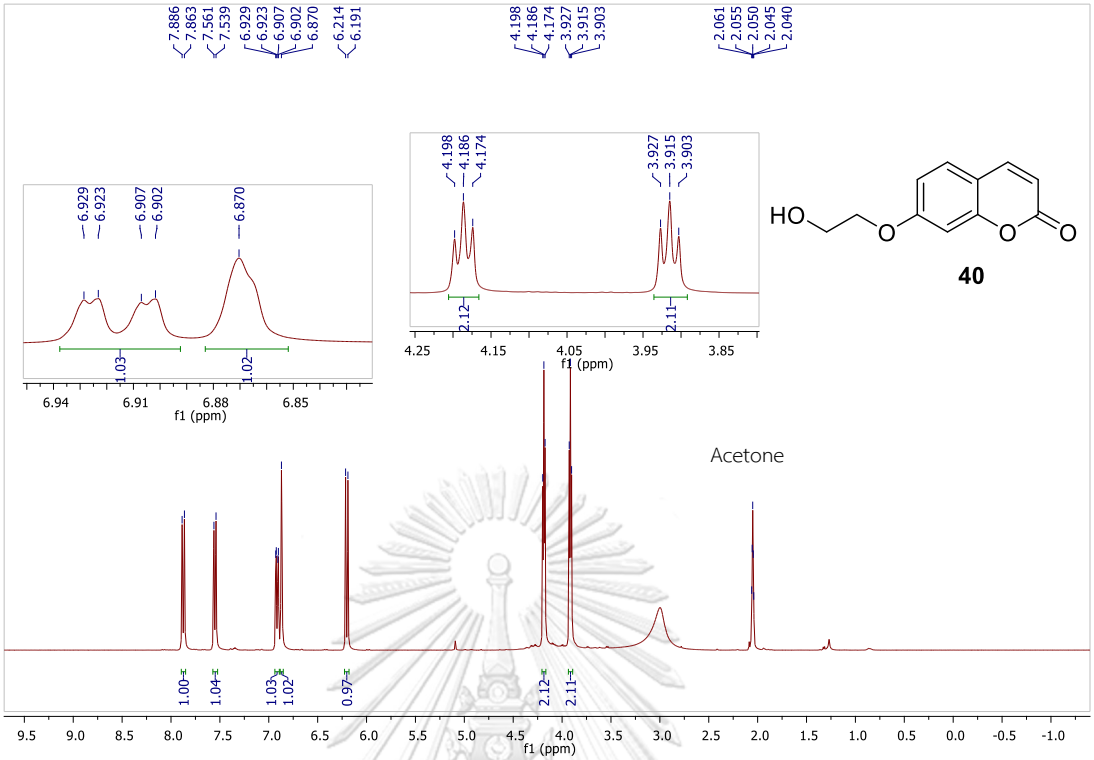


Figure A.21 ^{1}H-NMR (Acetone-d_6, 400 MHz) of **40.**

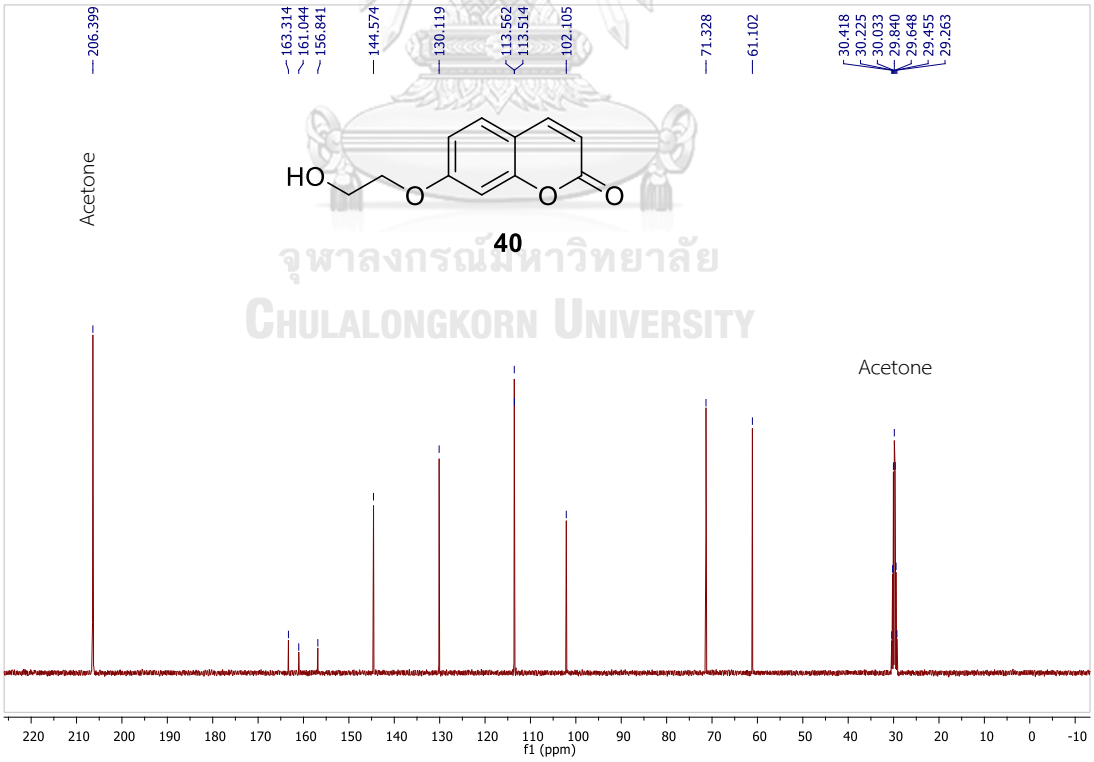


Figure A.22 ^{13}C-NMR (Acetone-d_6, 400 MHz) of **40.**

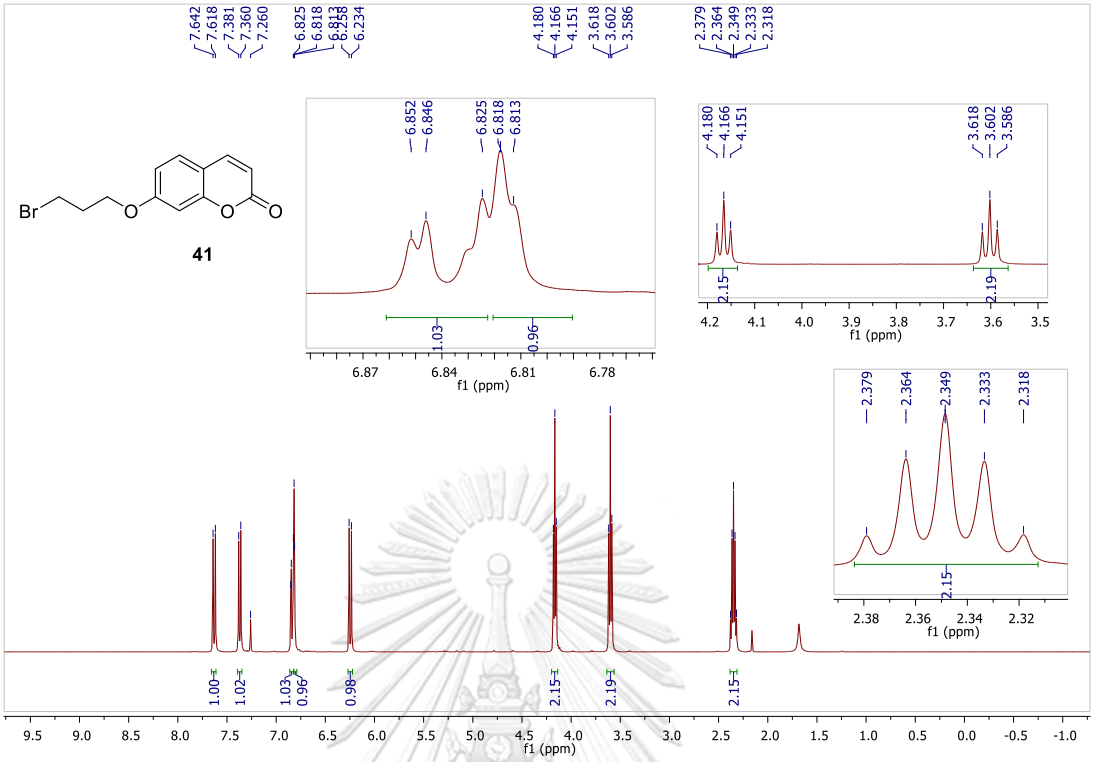


Figure A.23 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **41.**

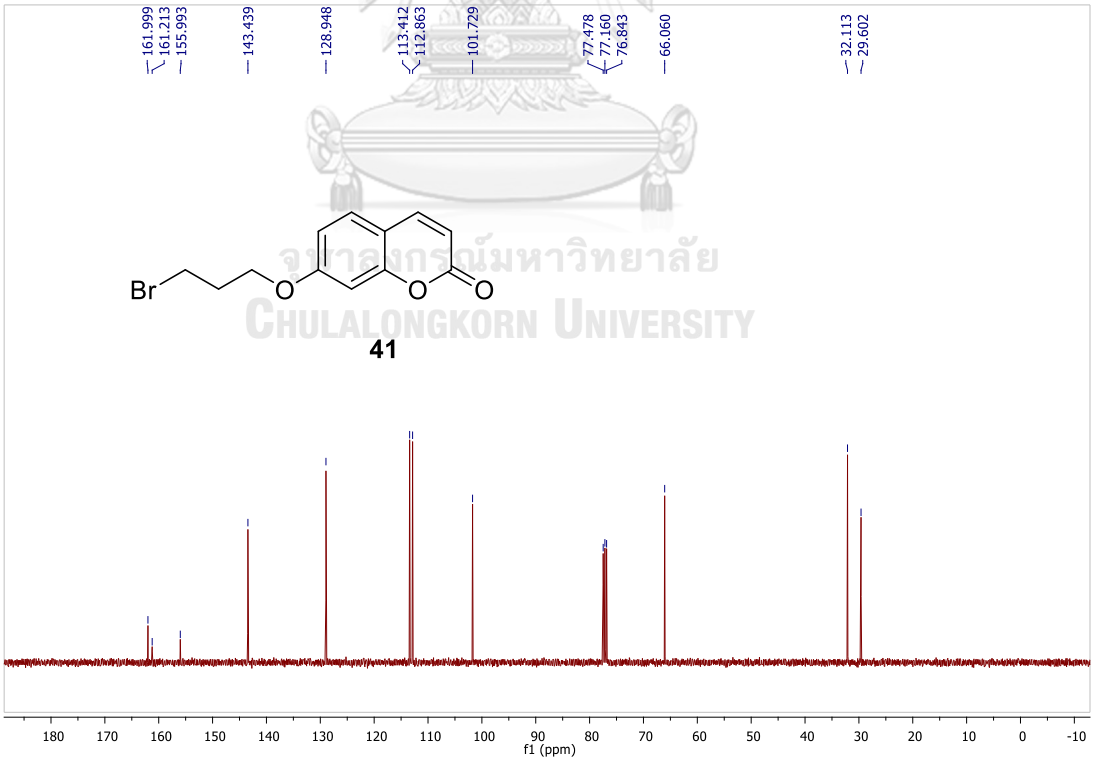


Figure A.24 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **41.**

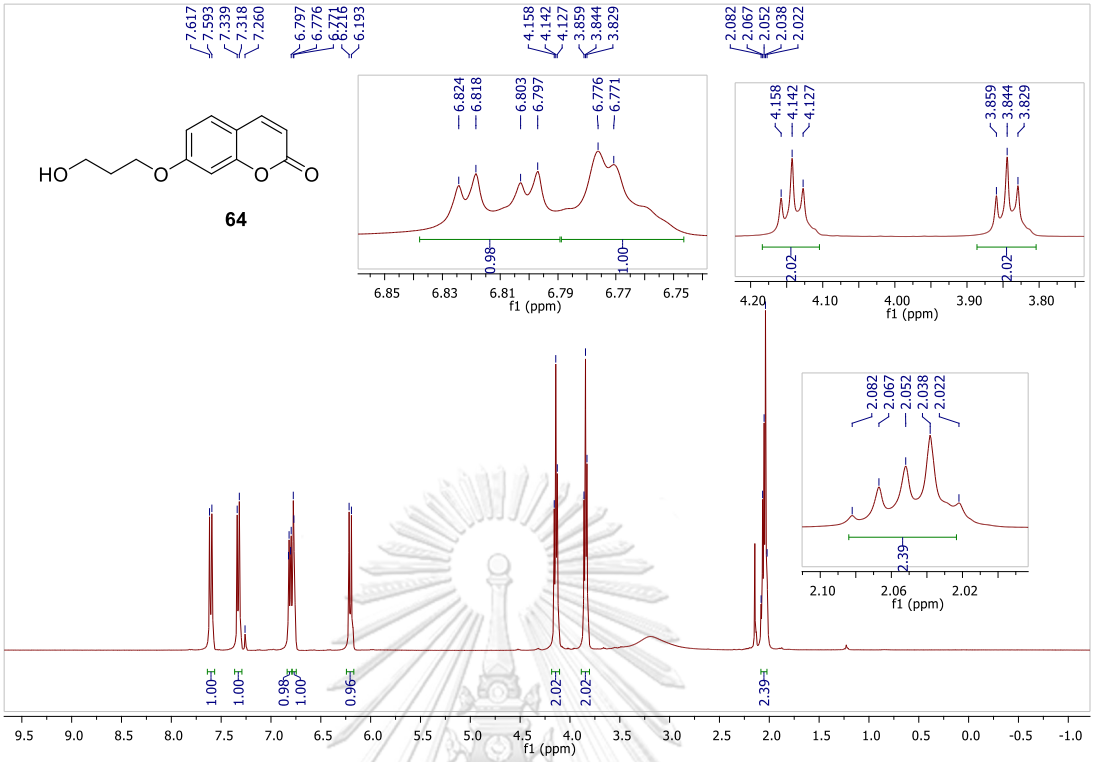


Figure A.25 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **64.**

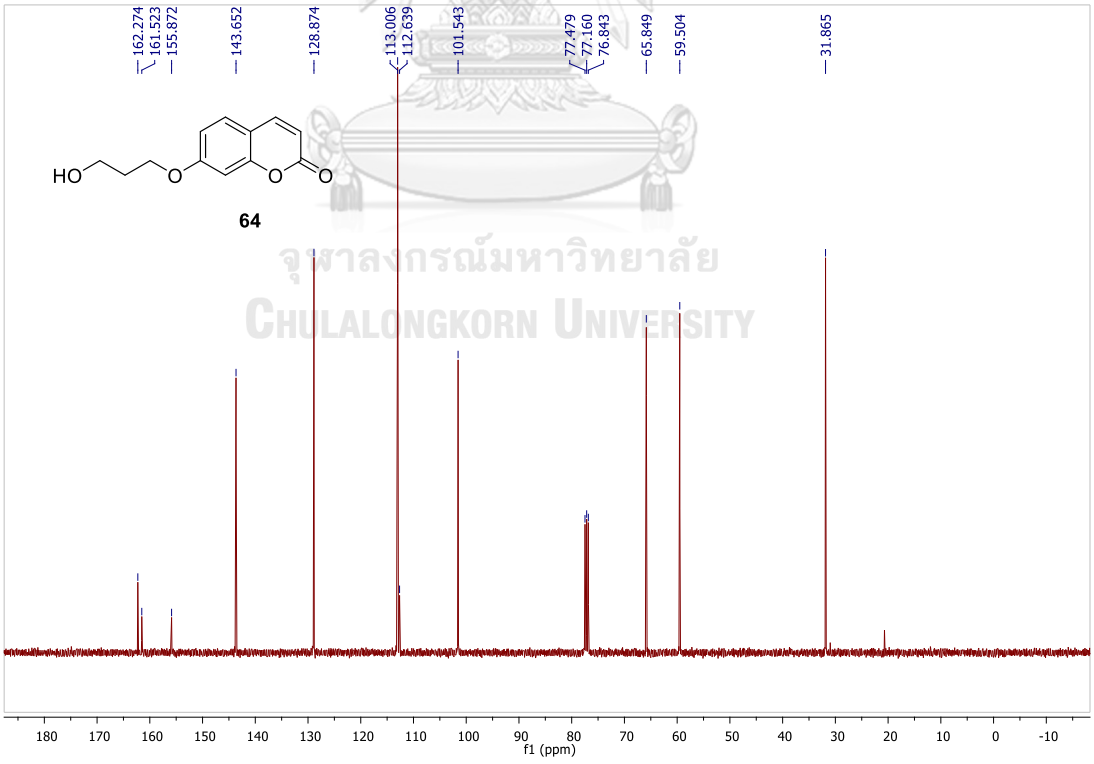


Figure A.26 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **64.**

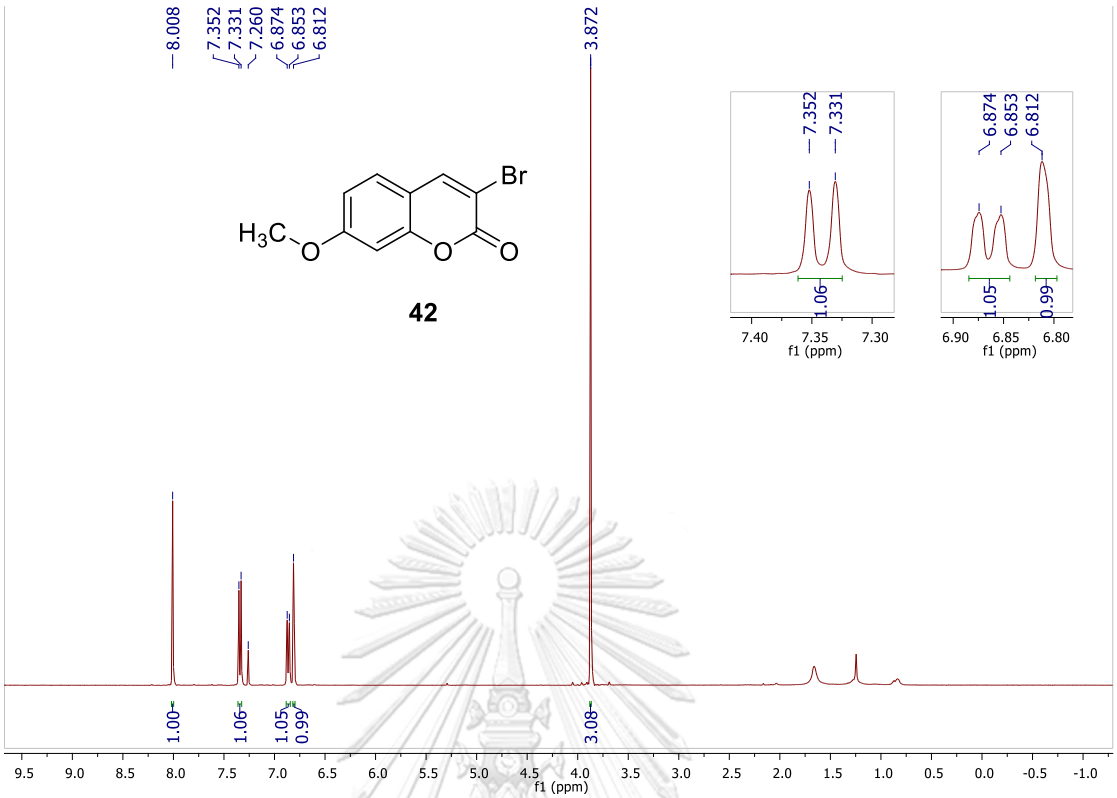


Figure A.27 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **42.**

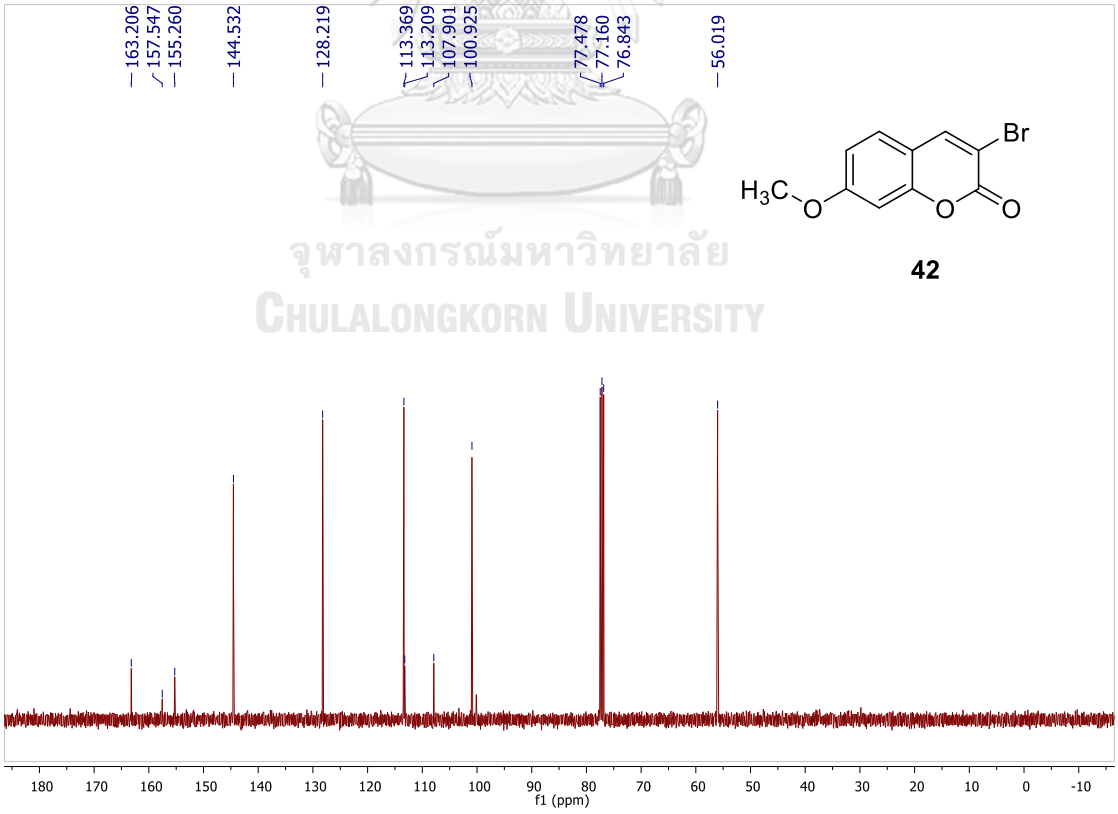


Figure A.28 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **42.**

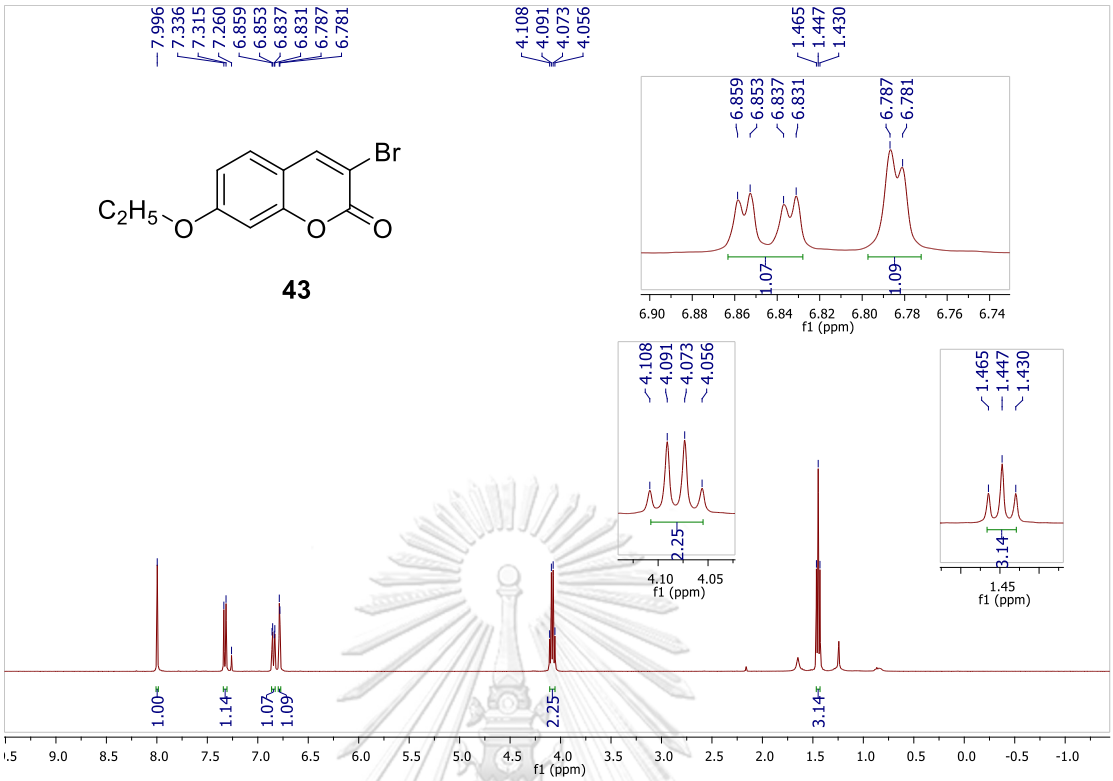


Figure A.29 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **42.**

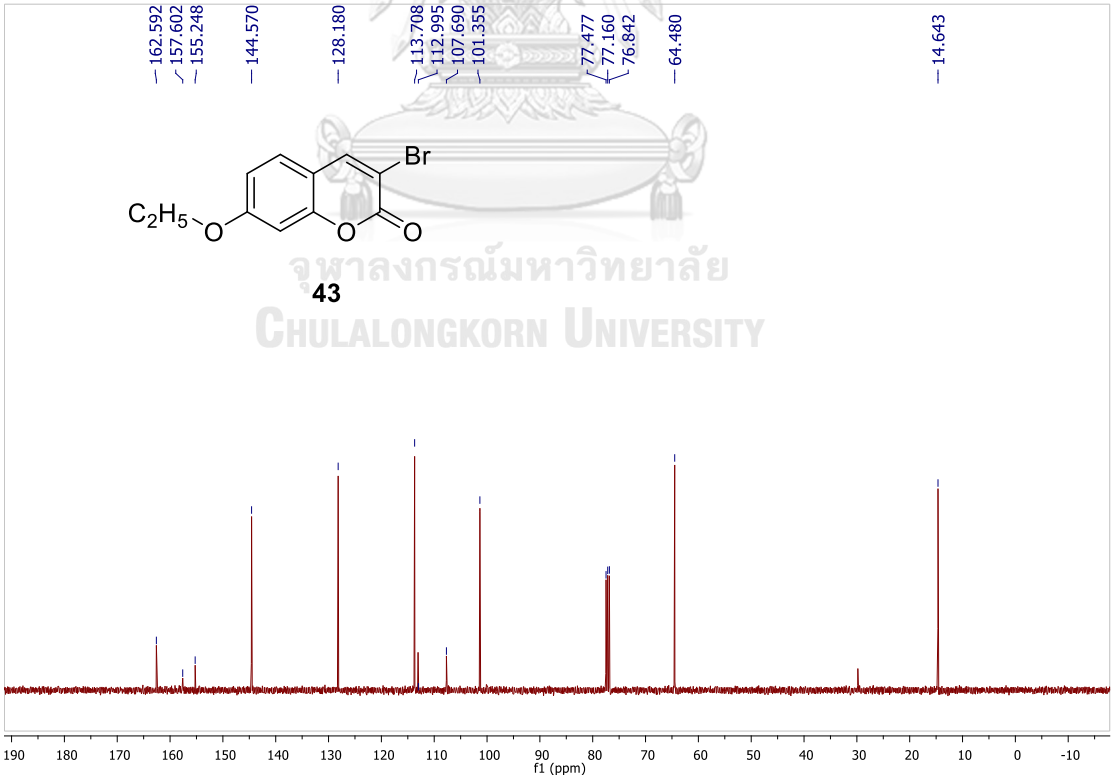


Figure A.30 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **43.**

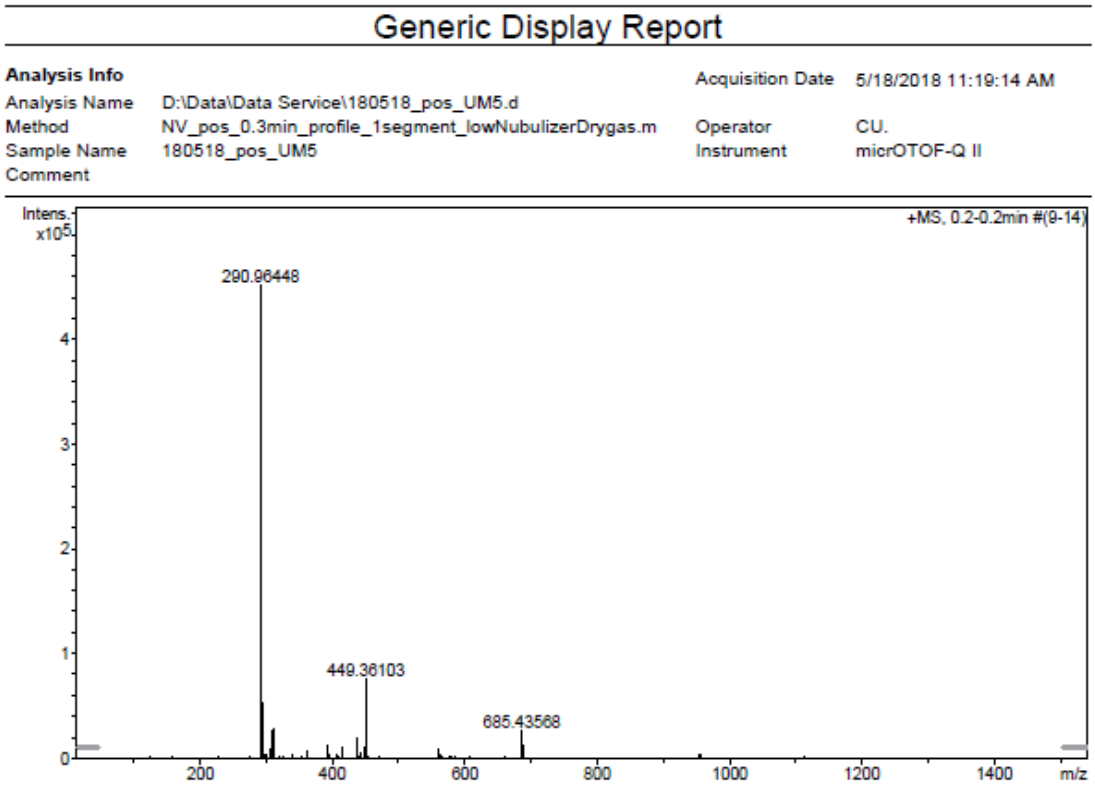


Figure A.31 HRMS of **43.**

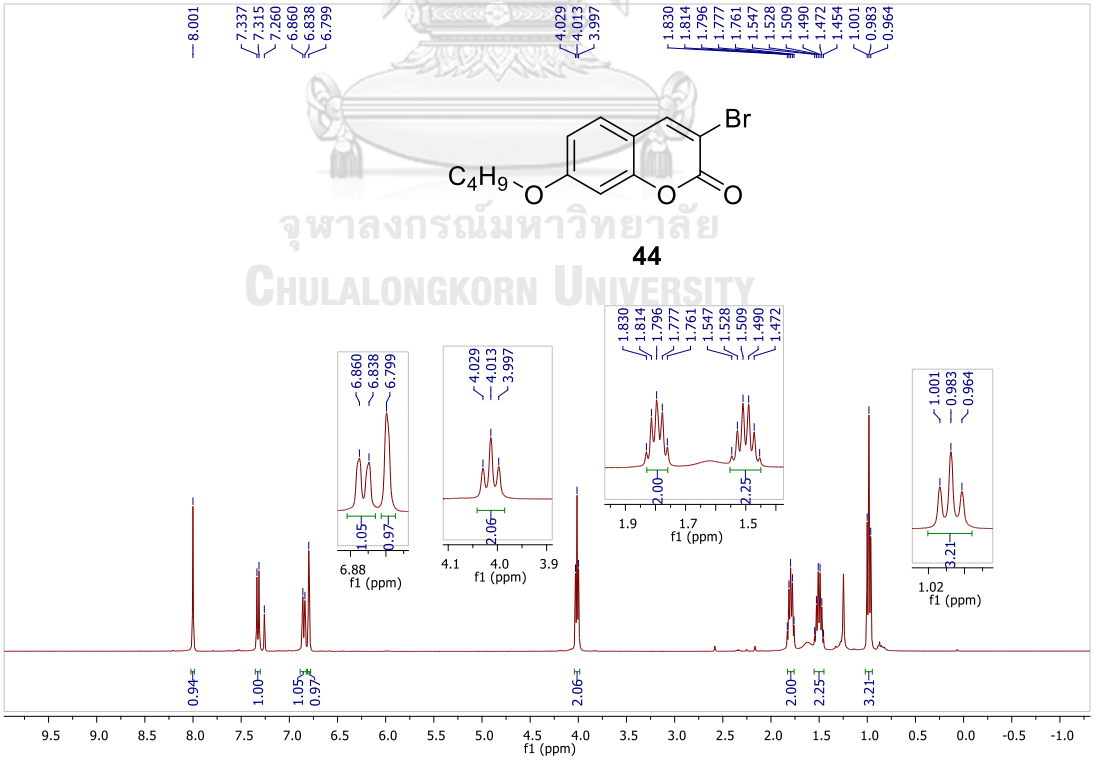


Figure A.32 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **44.**

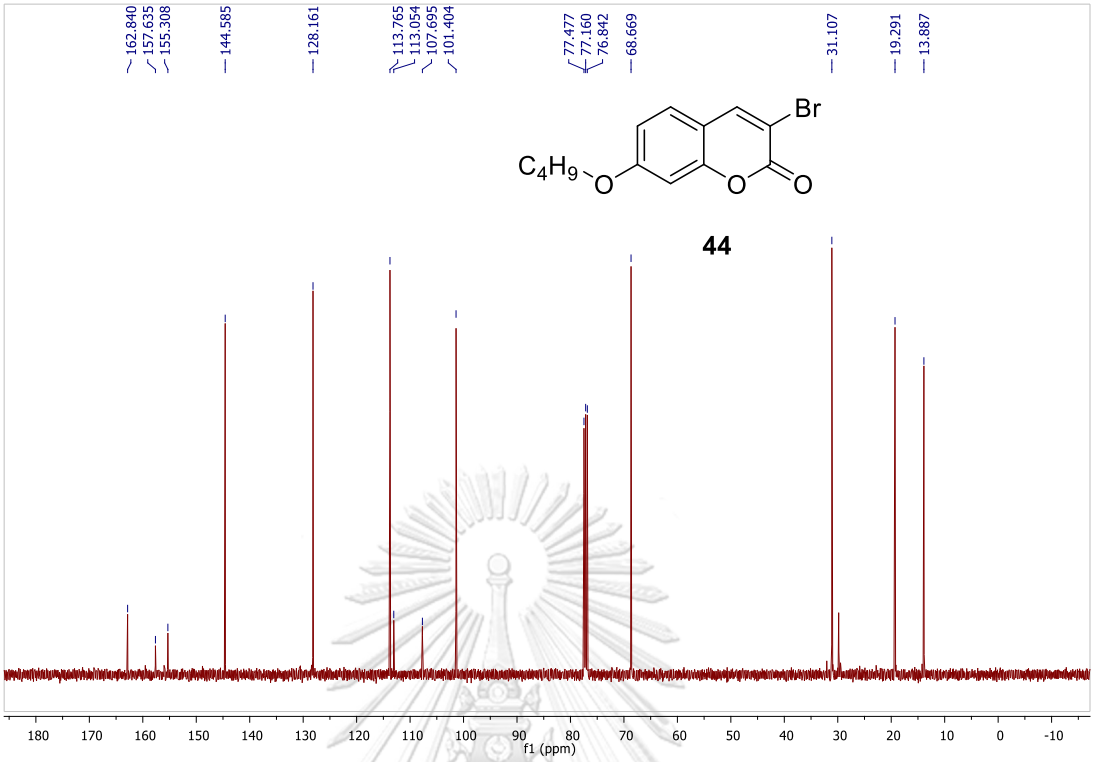


Figure A.33 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **44**.

Generic Display Report

Analysis Info

Analysis Name	D:\Data\Data Service\190930\UM26_RA3_01_3123.d	Acquisition Date	9/30/2019 5:05:09 PM
Method	nv_pos_6min_profile_wguardcol_190624.m	Operator	CU.
Sample Name	UM26	Instrument	micrOTOF-Q II
Comment			

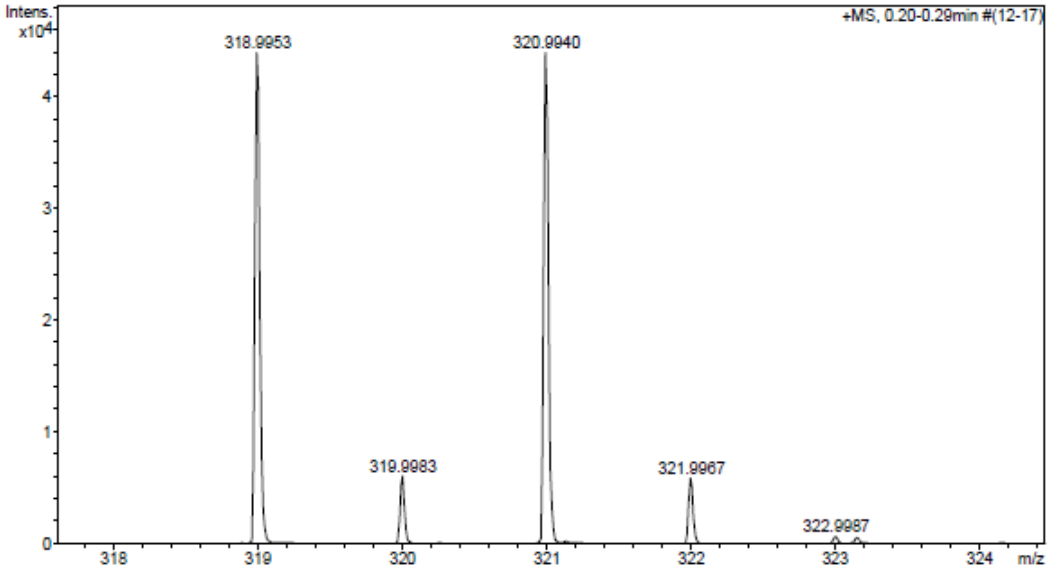


Figure A.34 HRMS of **44**.

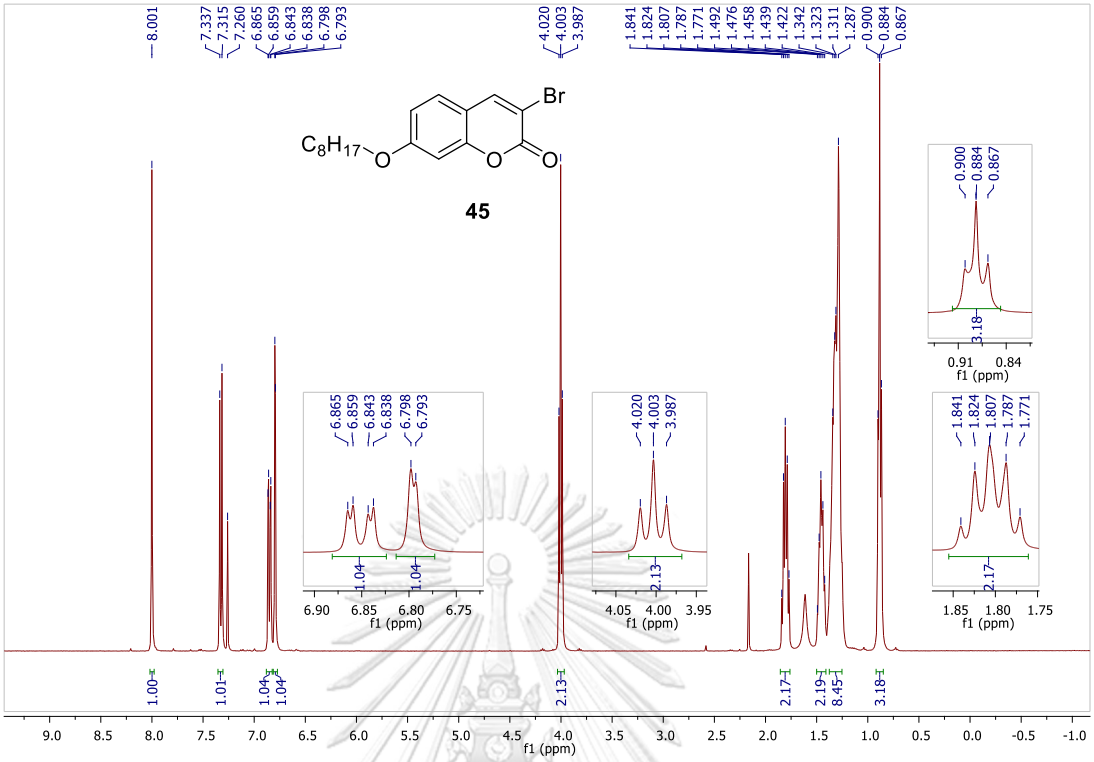


Figure A.35 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **45.**

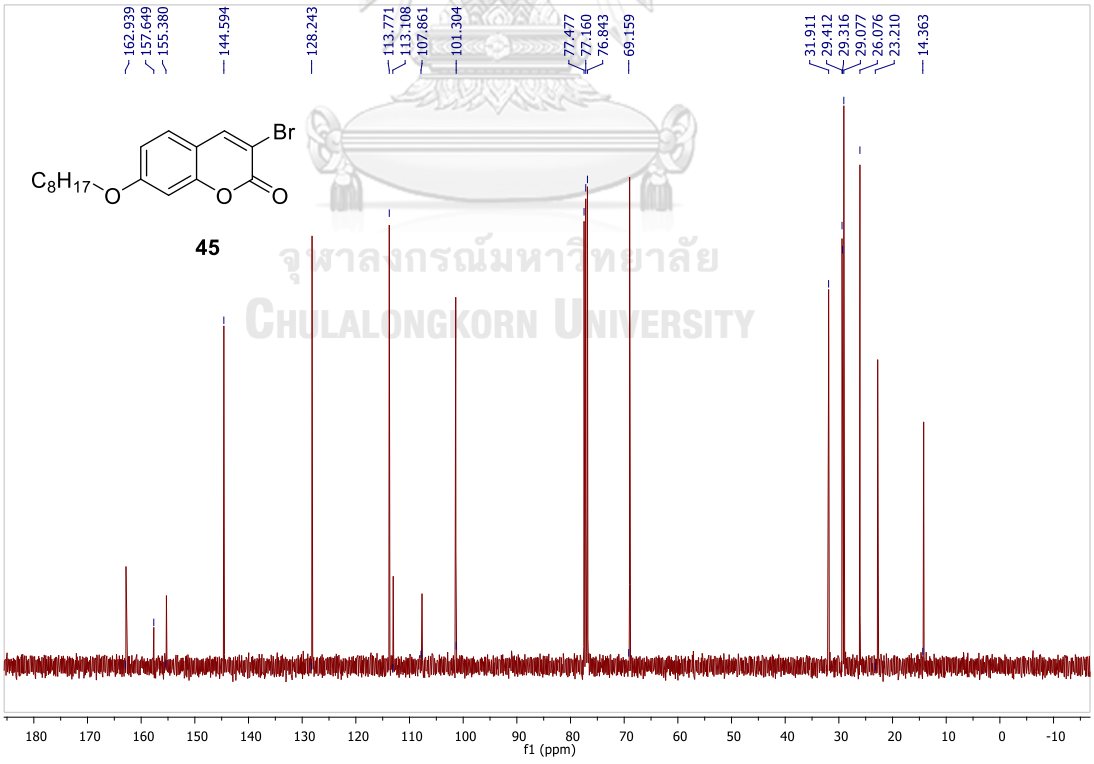


Figure A.36 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **45.**

Generic Display Report

Analysis Info

Analysis Name	D:\Data\Data Service\190930\UM27_RA2_01_3122.d	Acquisition Date	9/30/2019 4:58:52 PM
Method	nv_pos_6min_profile_wguardcol_190624.m	Operator	CU.
Sample Name	UM27	Instrument	micrOTOF-Q II
Comment			

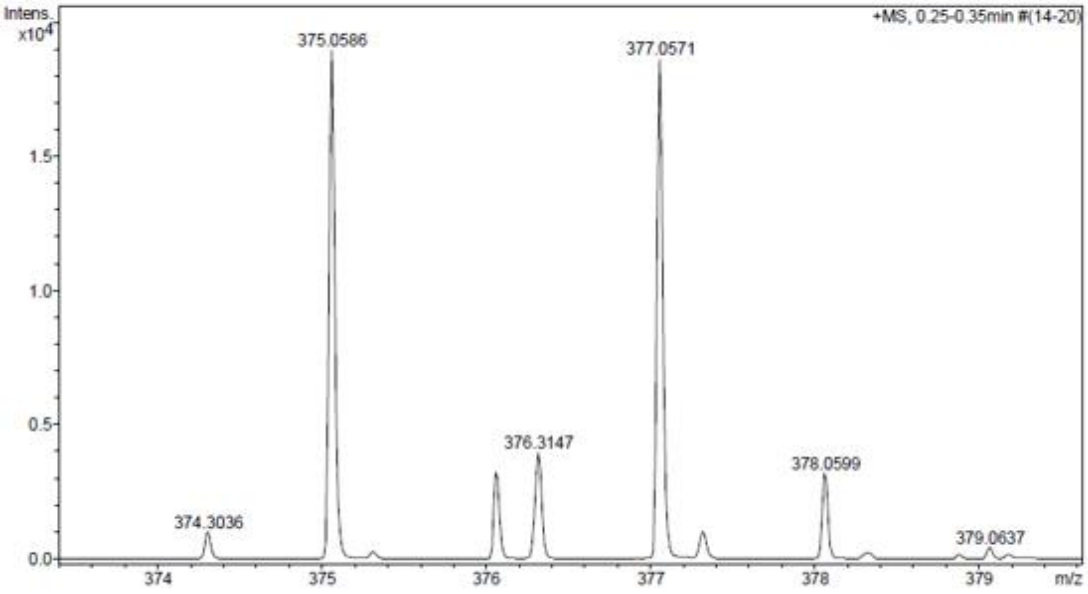


Figure A.37 HRMS of **45.**

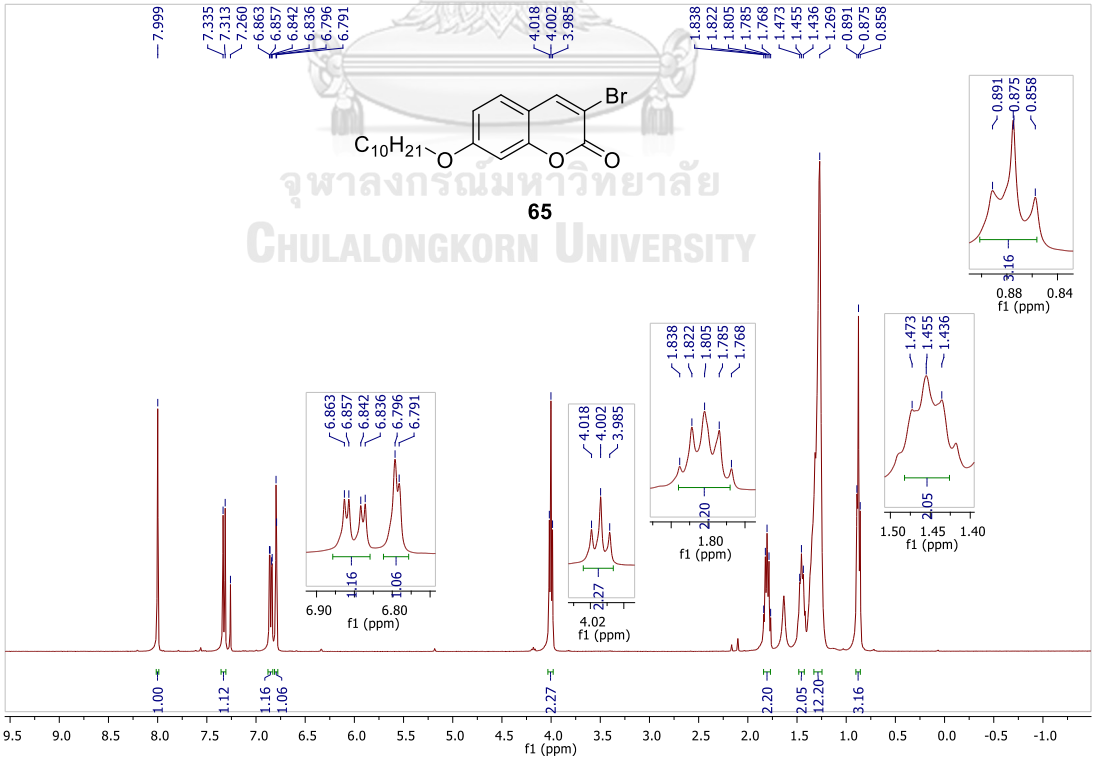


Figure A.38 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **65.**

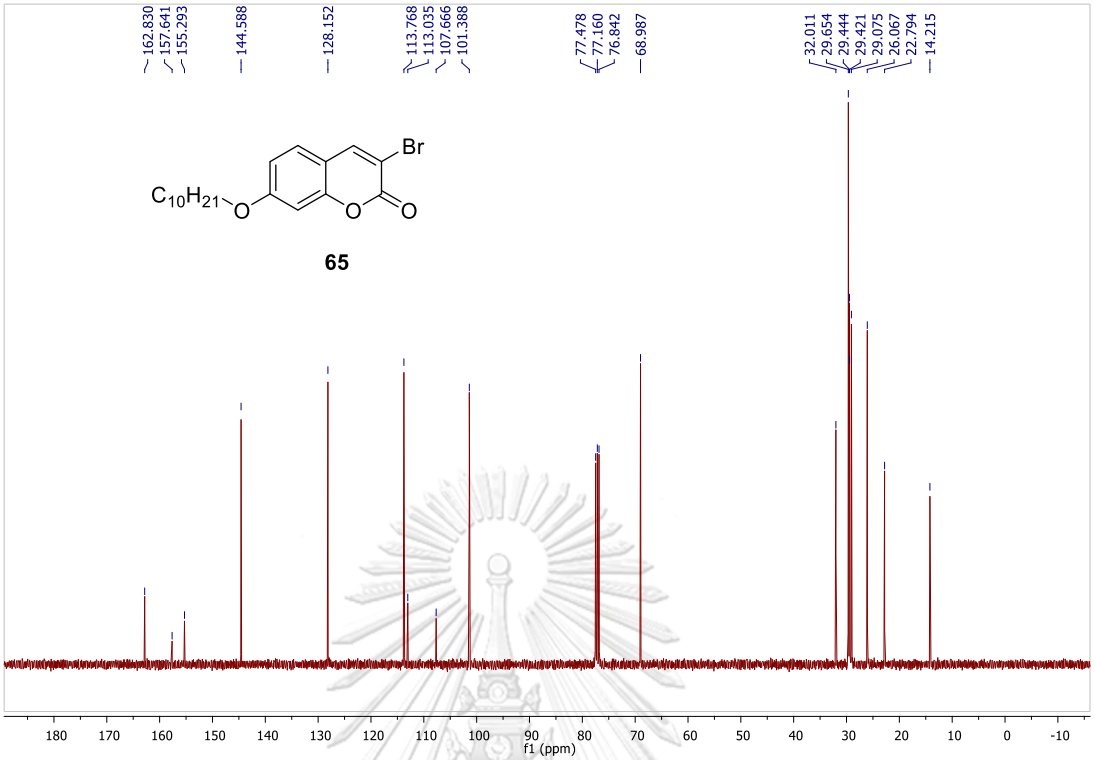


Figure A.39 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **65**.

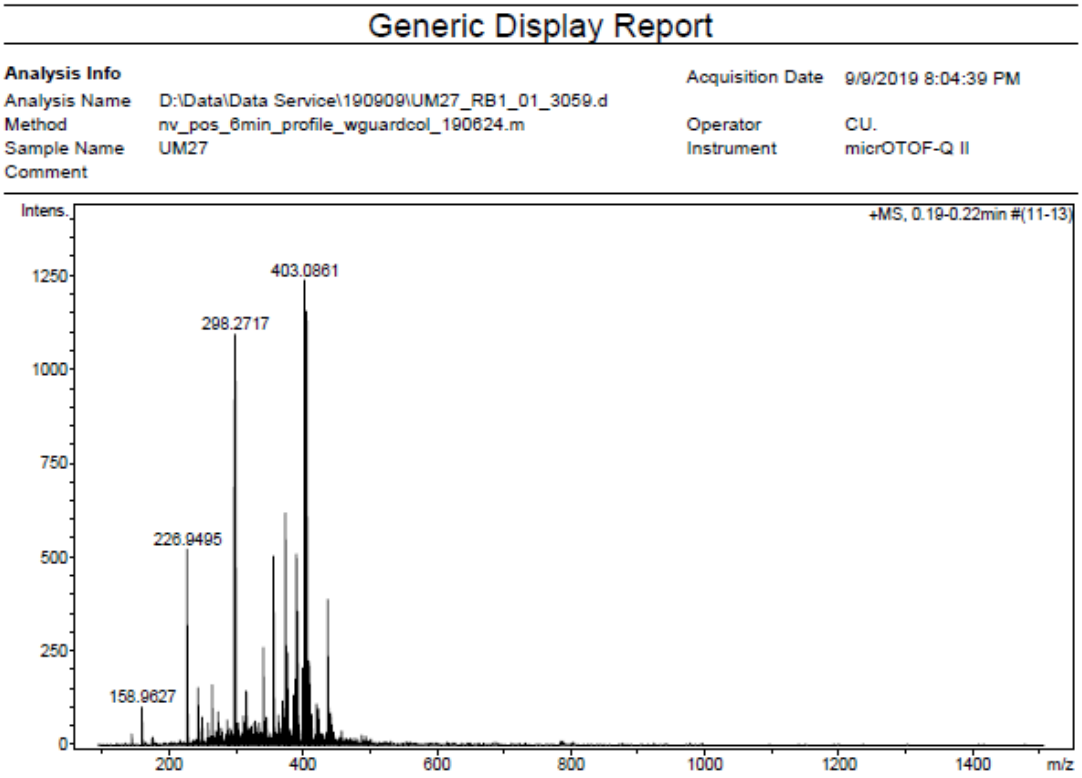


Figure A.40 HRMS of **65**.

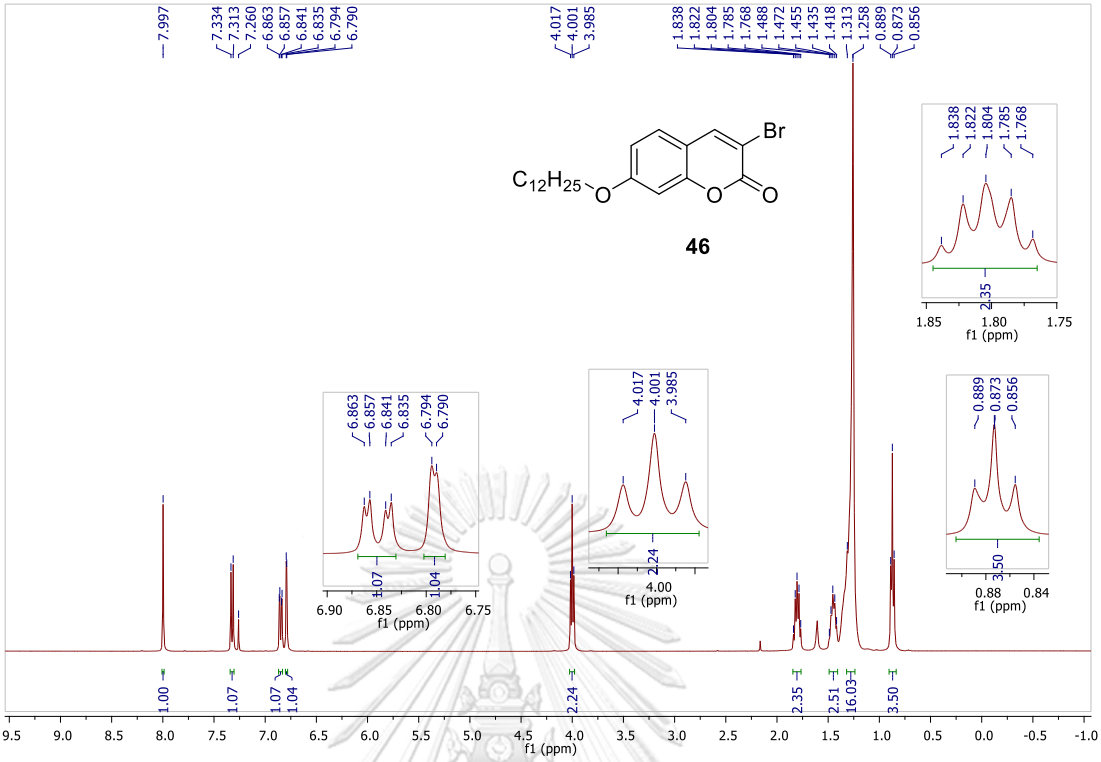


Figure A.41 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **46.**

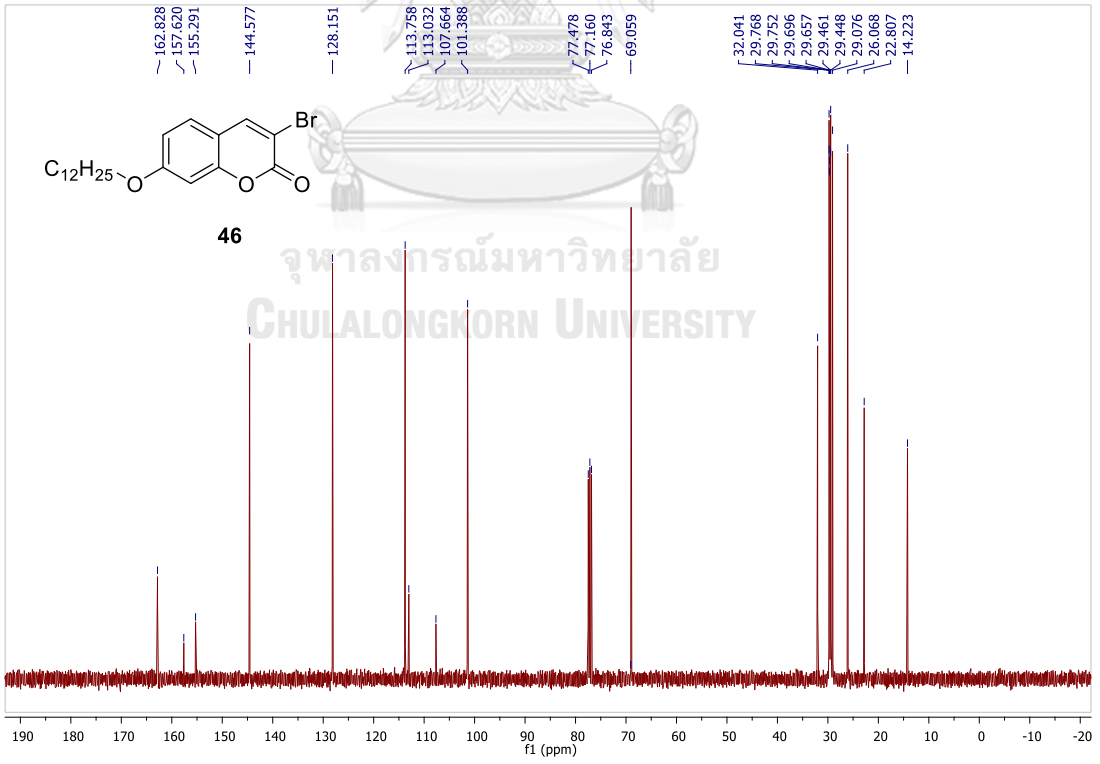


Figure A.42 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **46.**

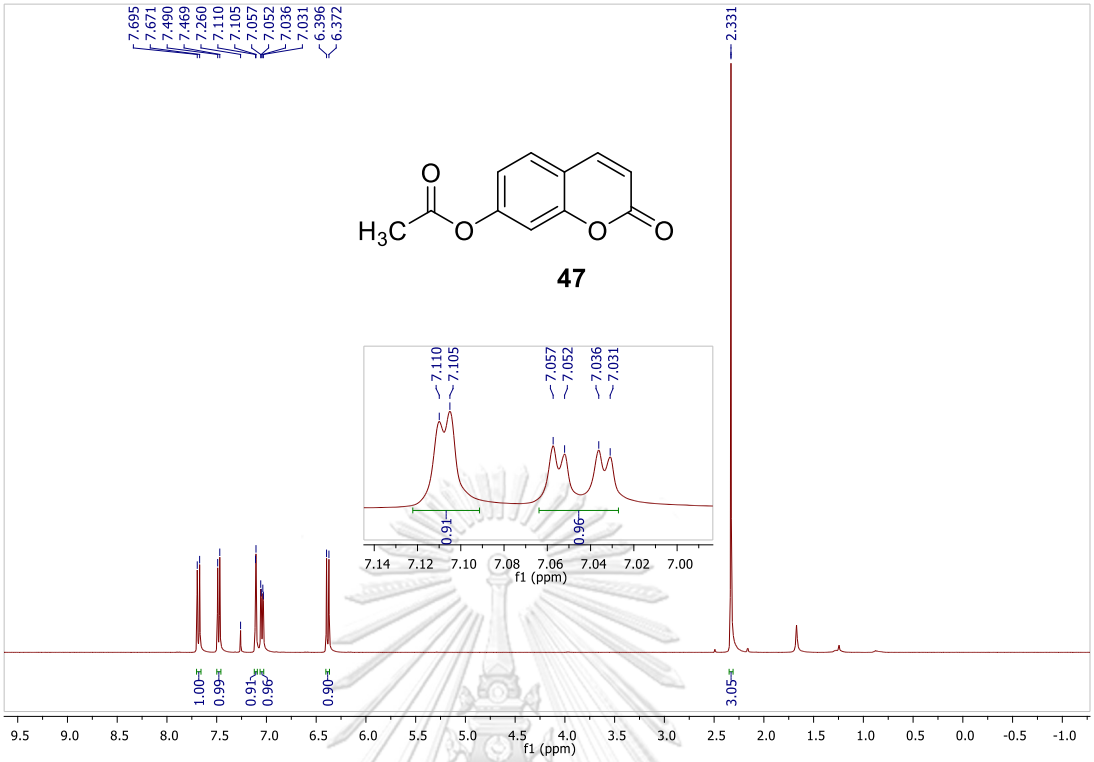


Figure A.43 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **47.**

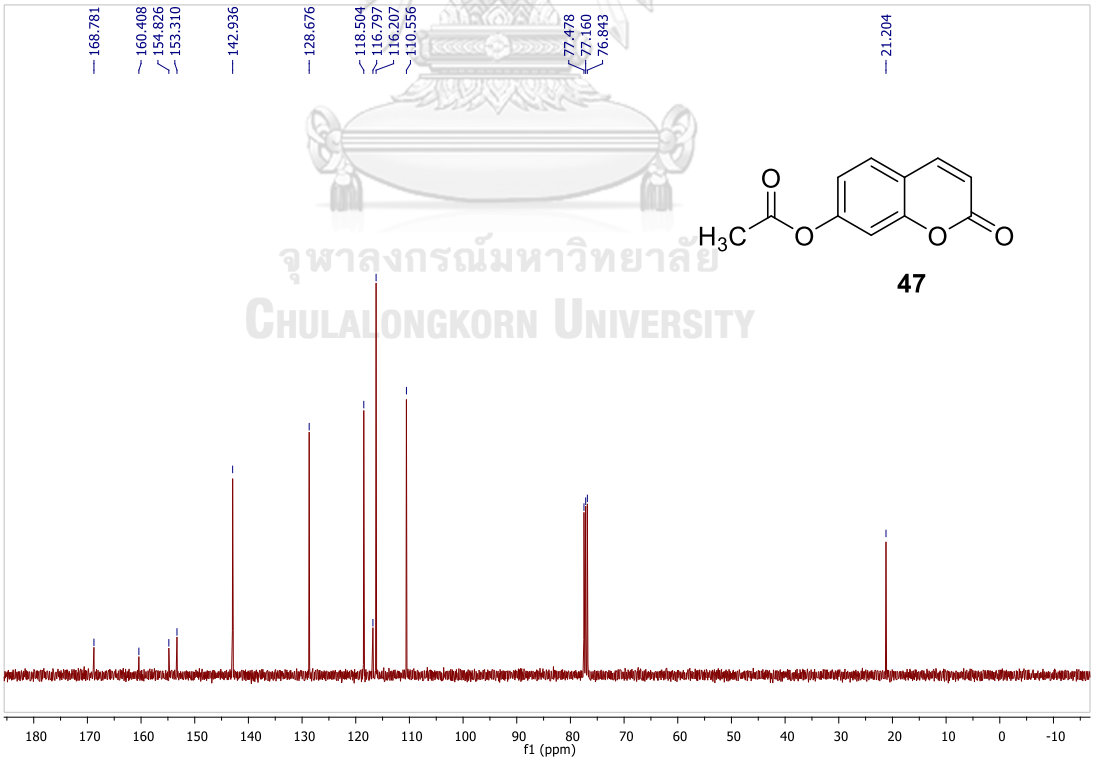


Figure A.44 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **47.**

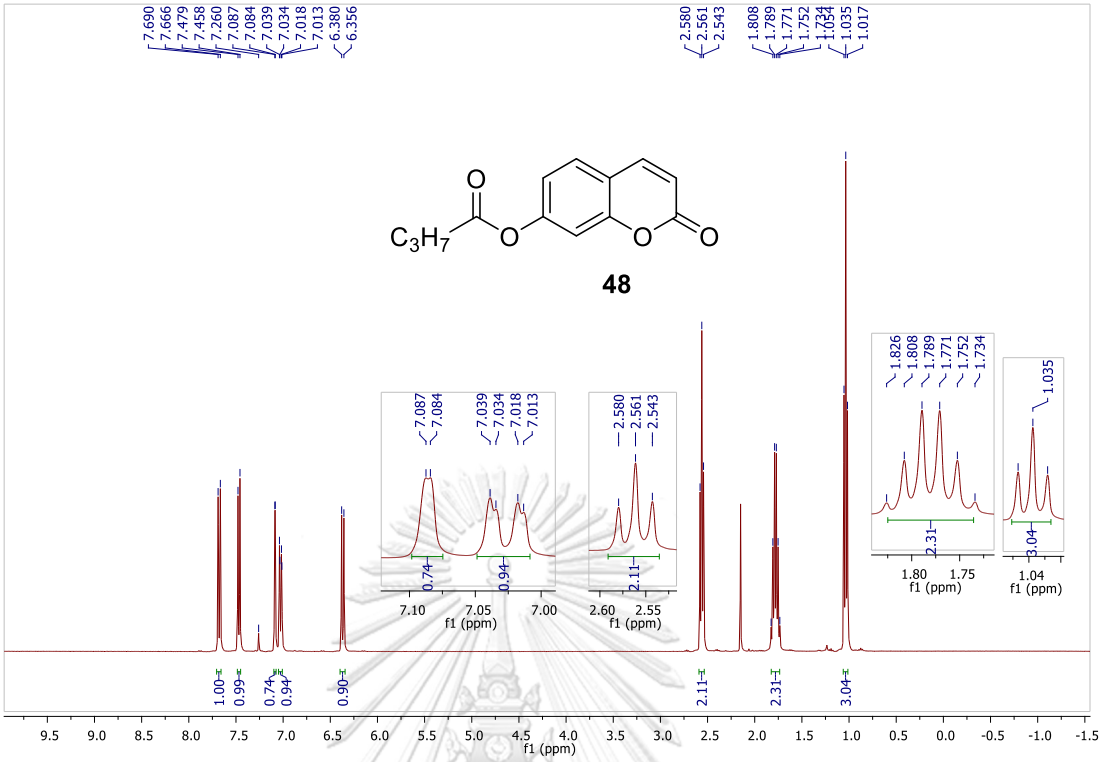


Figure A.45 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **48**.

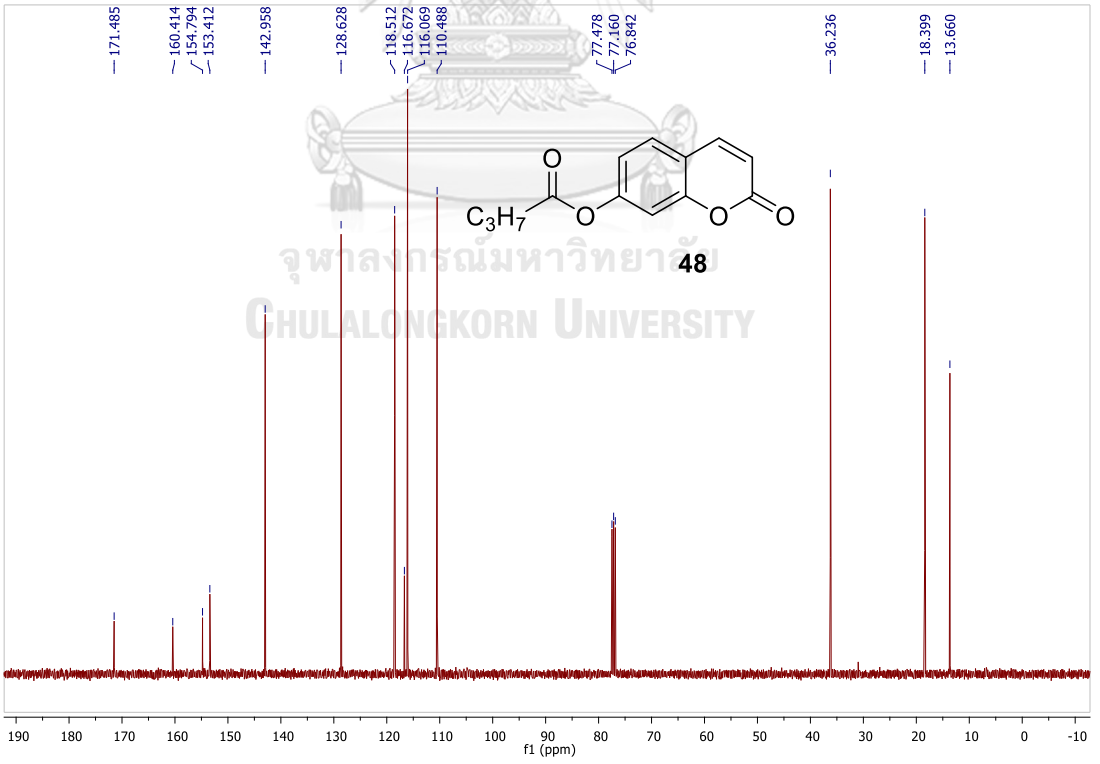


Figure A.46 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **48**.

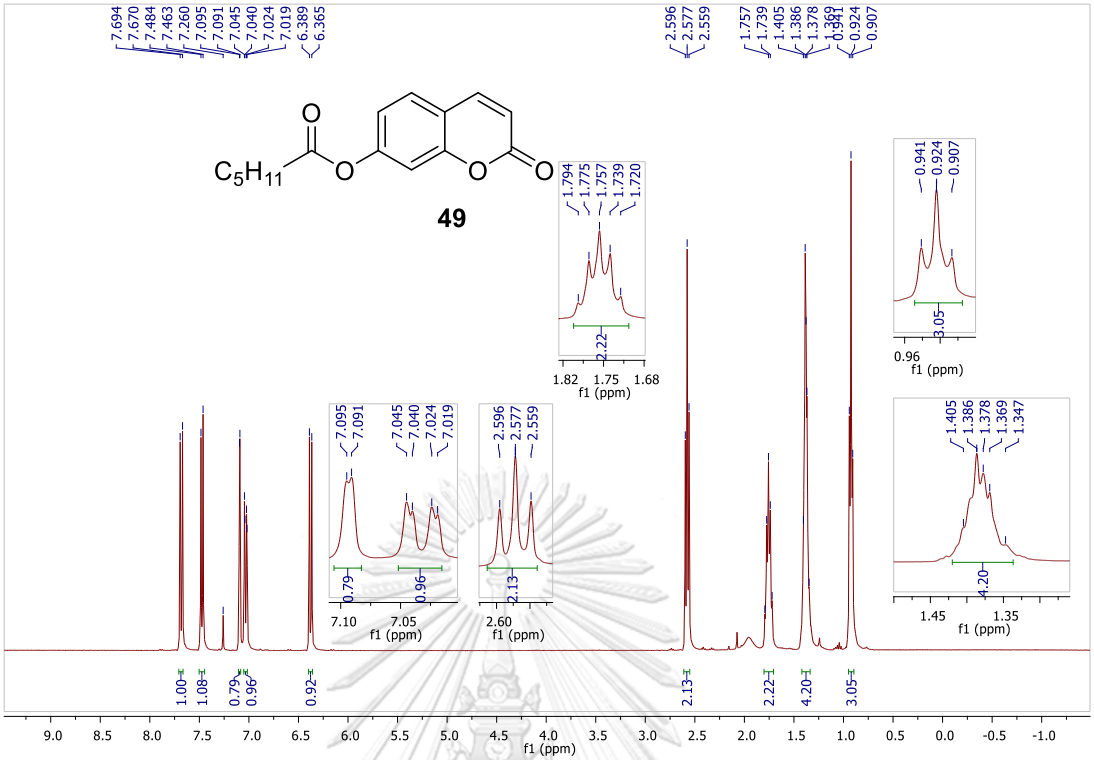


Figure A.47 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **49**.

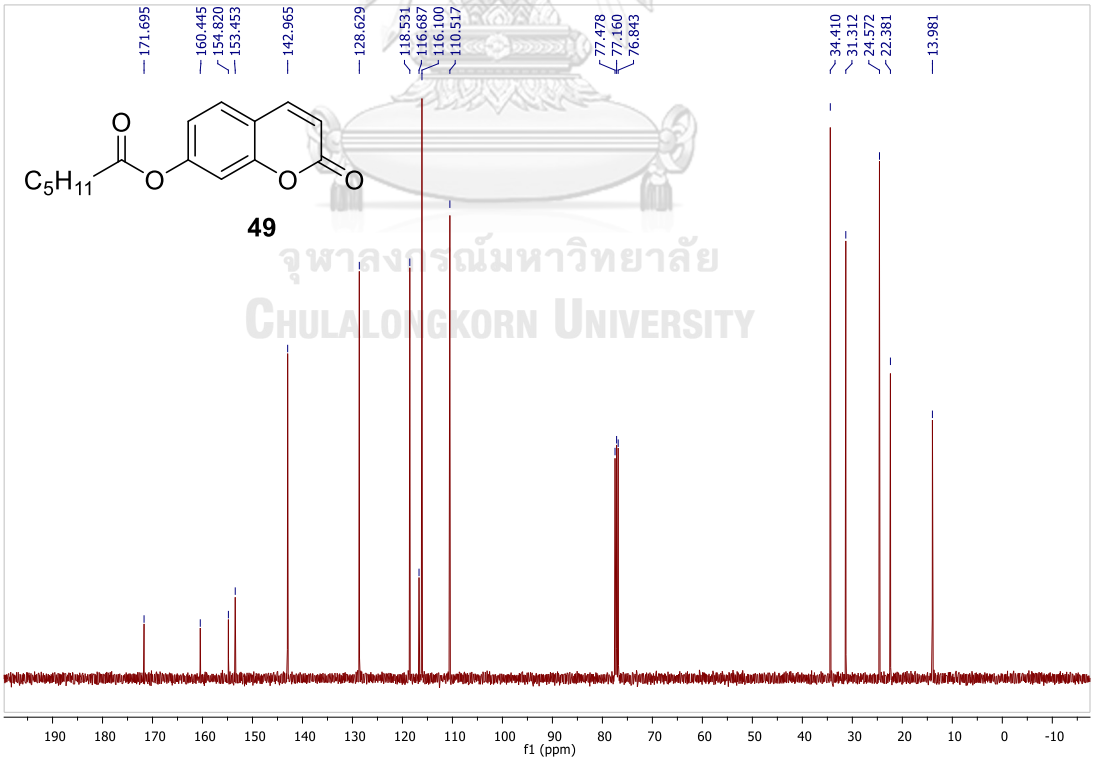


Figure A.48 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **49**.

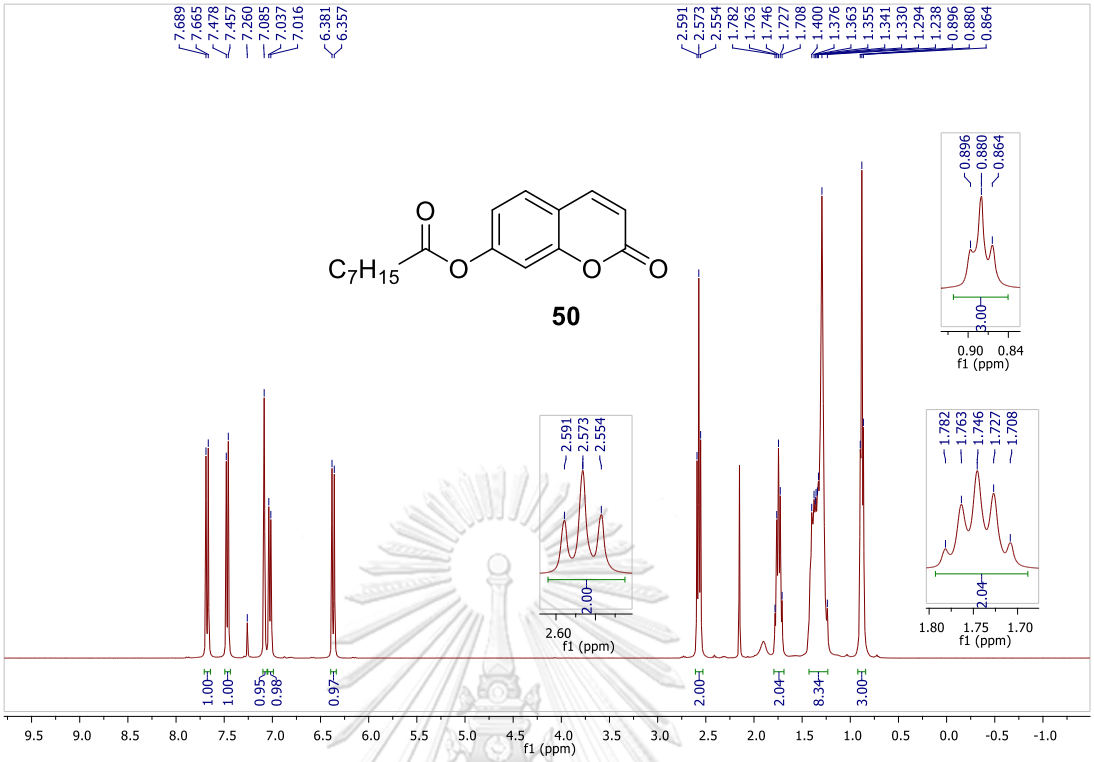


Figure A.49 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **50.**

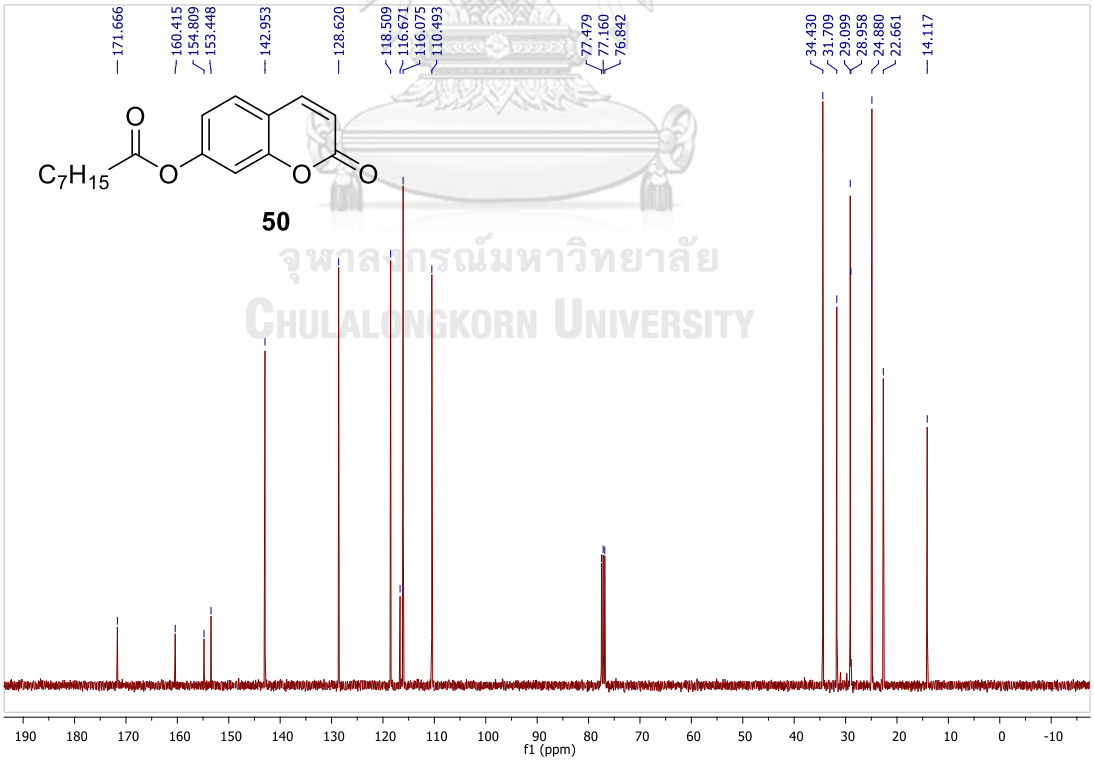


Figure A.50 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **50.**

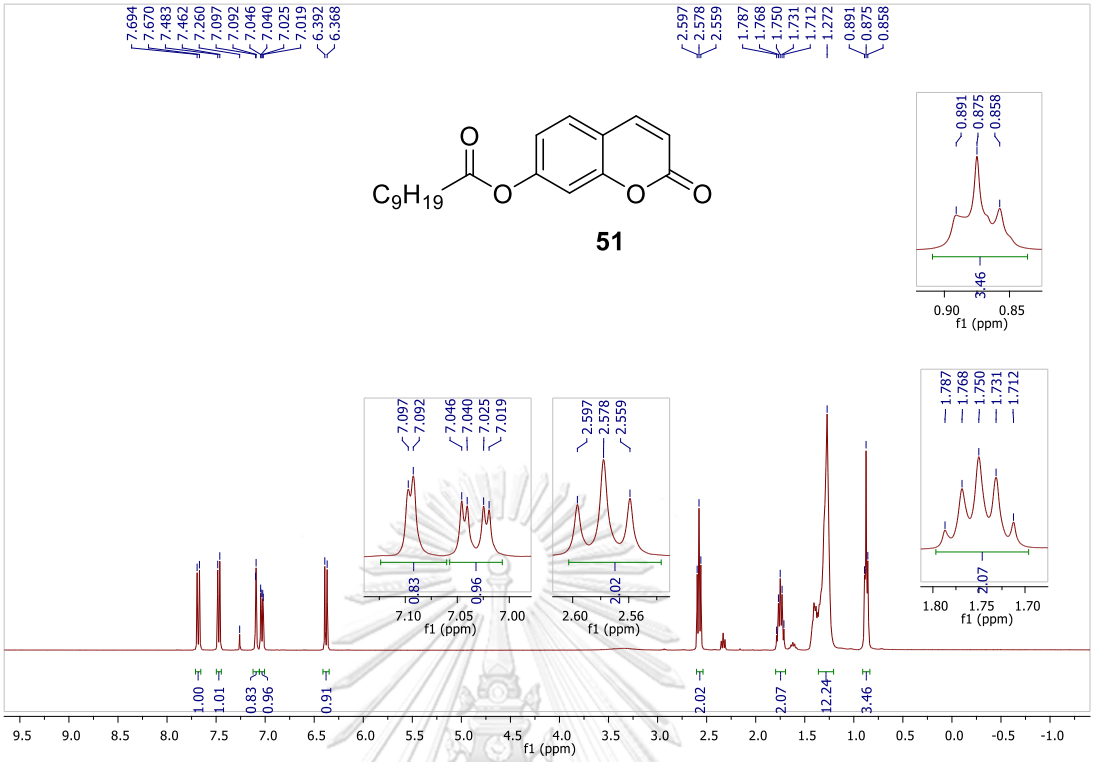


Figure A.51 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **51.**

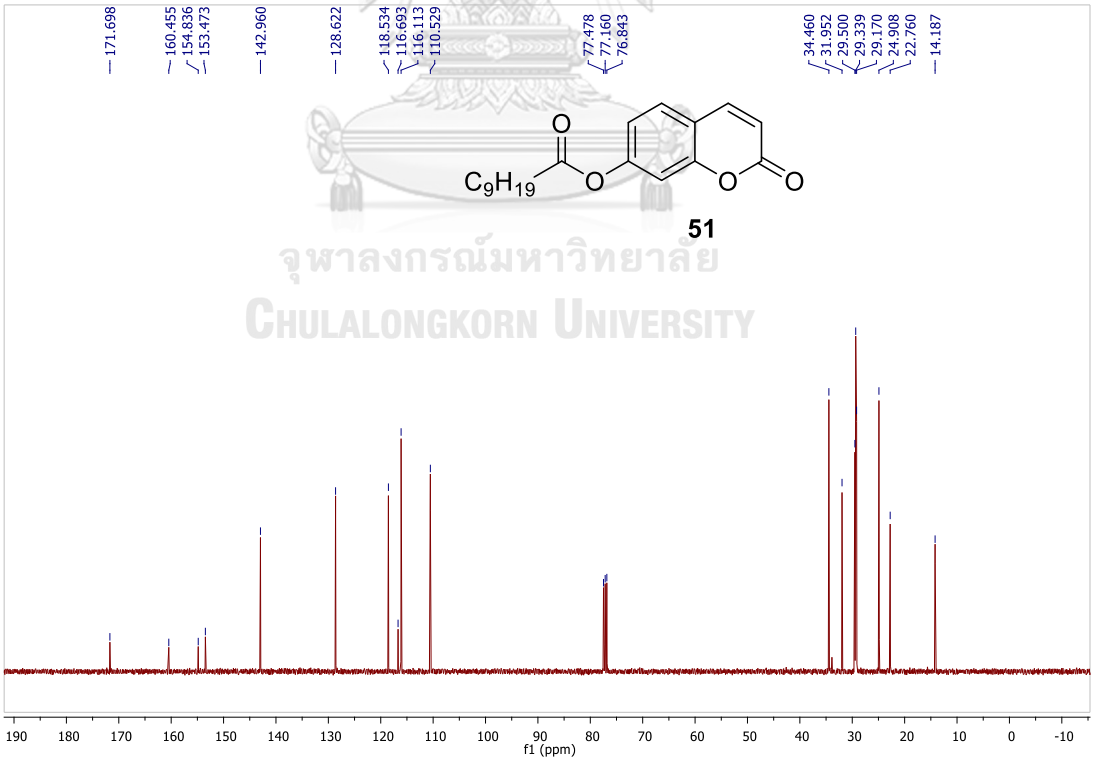


Figure A.52 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **51.**

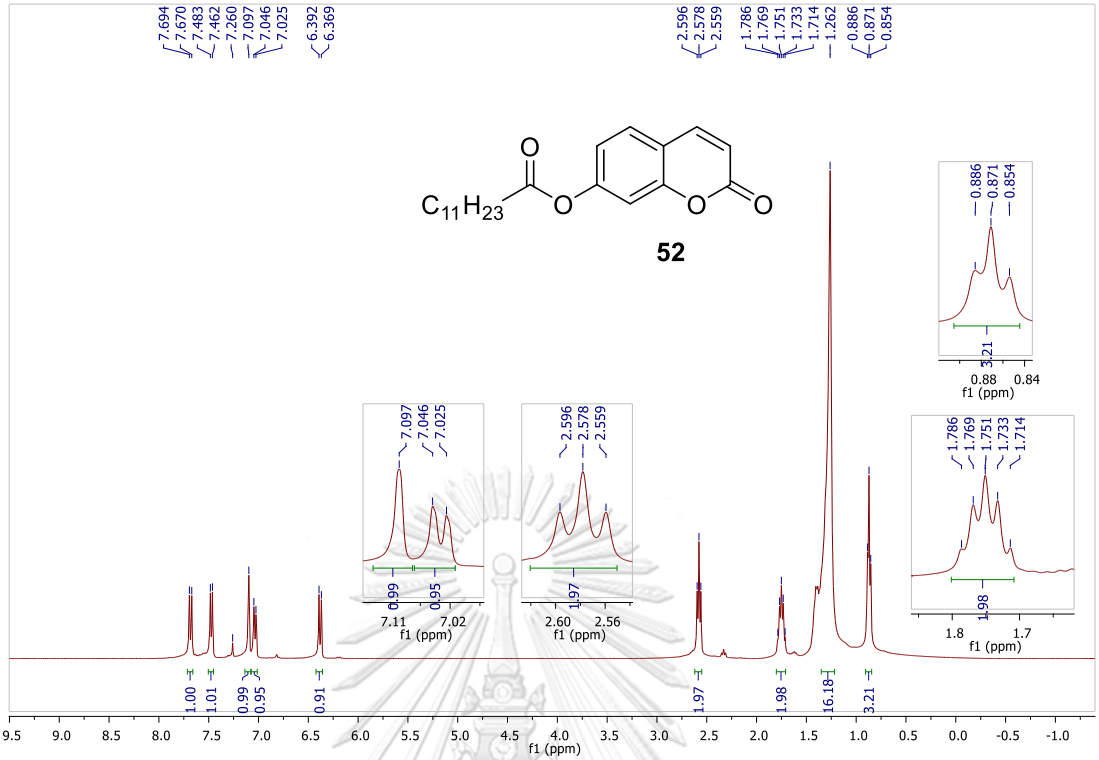


Figure A.53 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **52.**

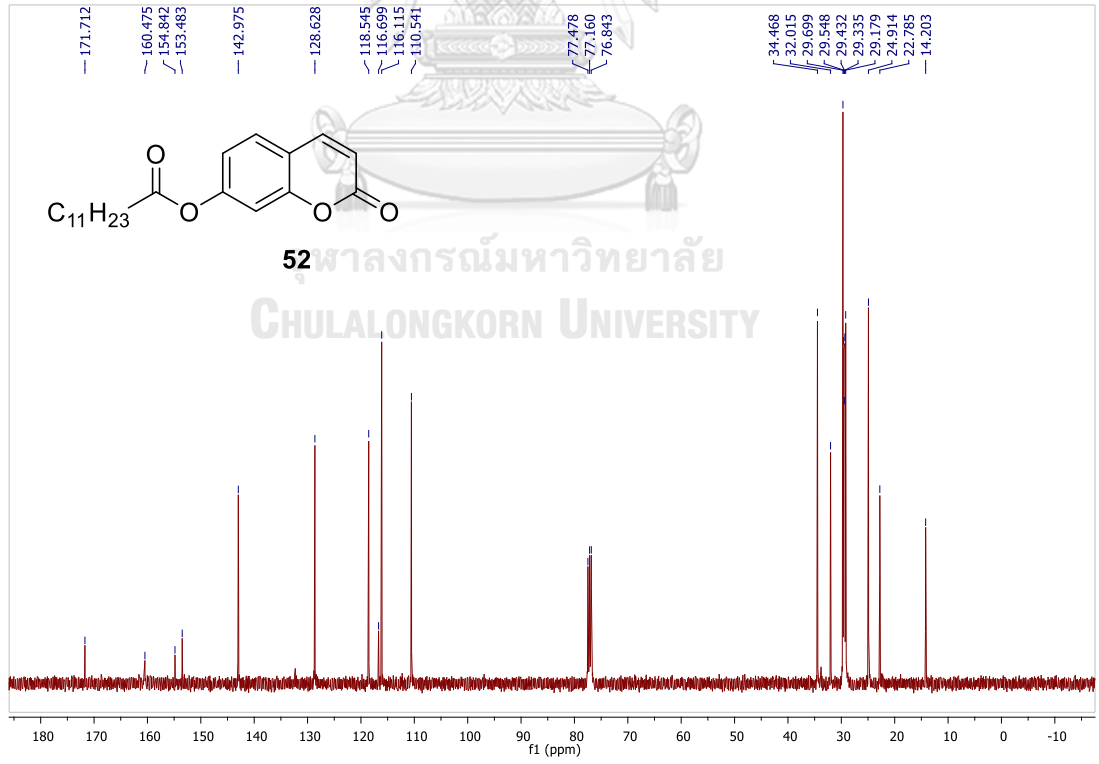


Figure A.54 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **52.**

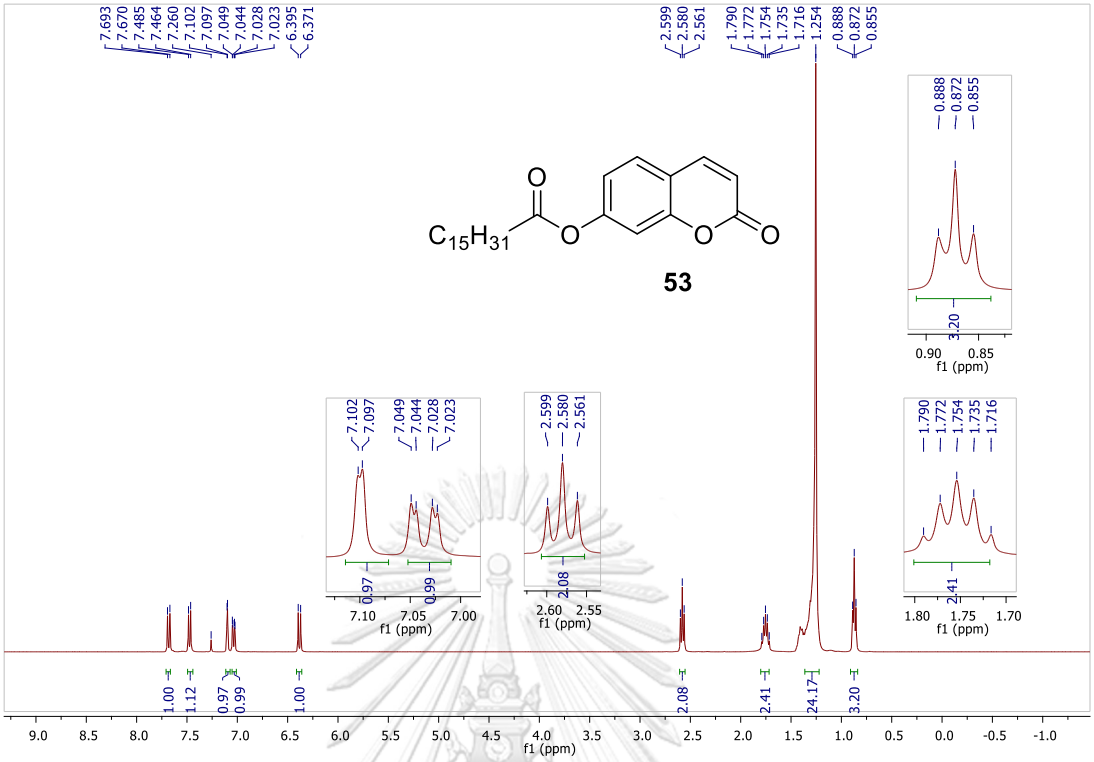


Figure A.55 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **53.**

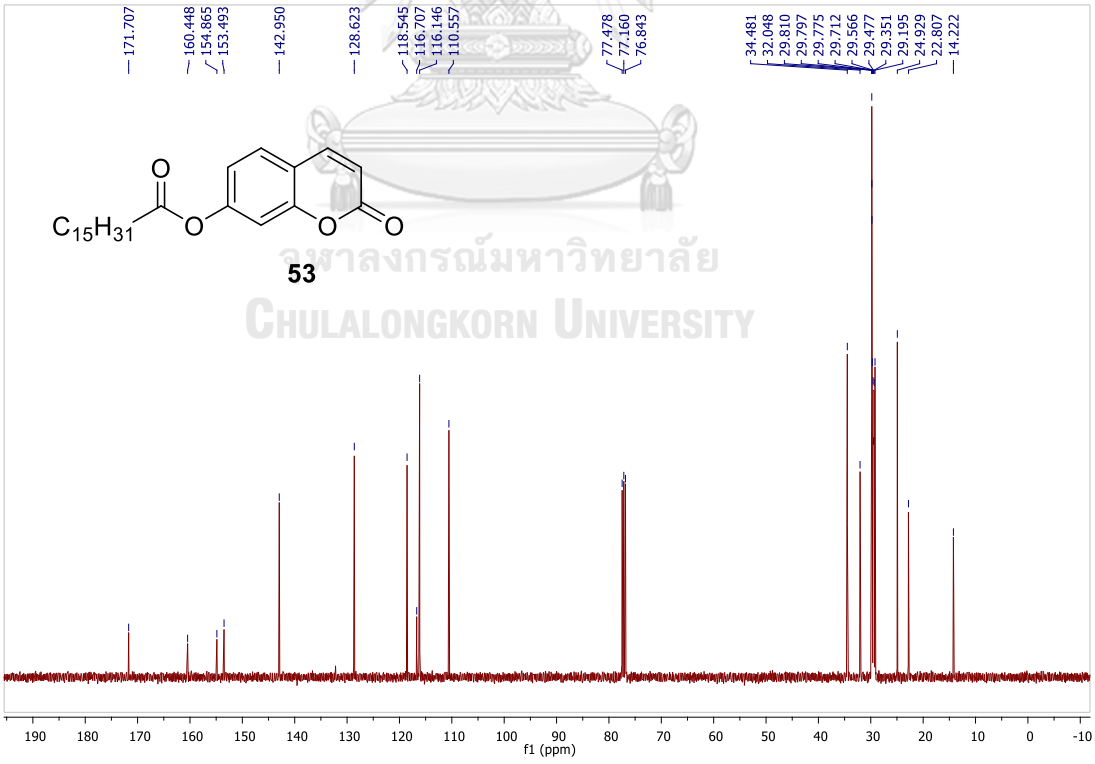


Figure A.56 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **53.**

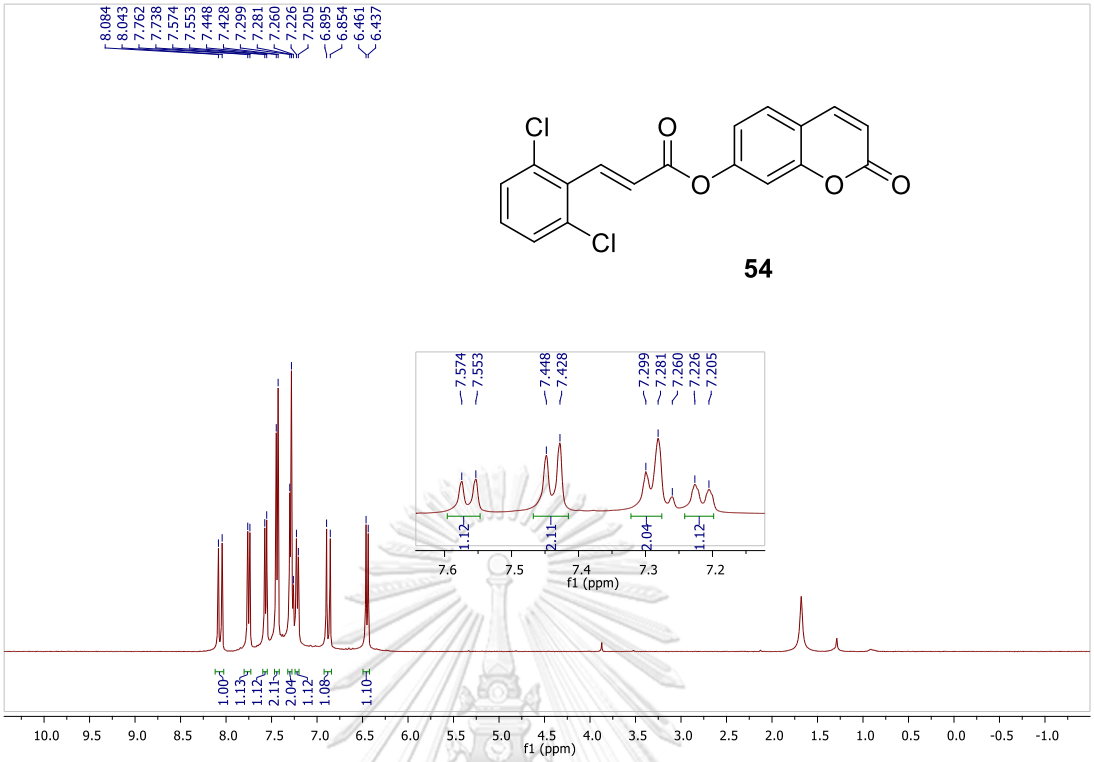


Figure A.57 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **54.**

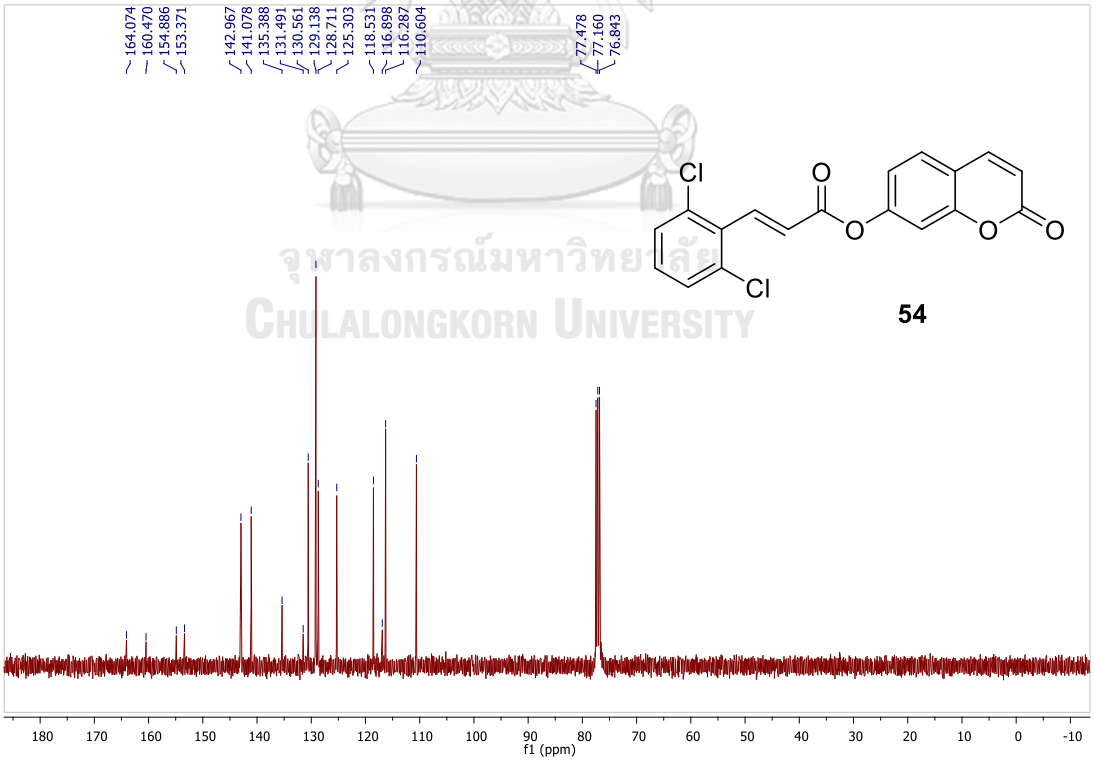


Figure A.58 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **54.**

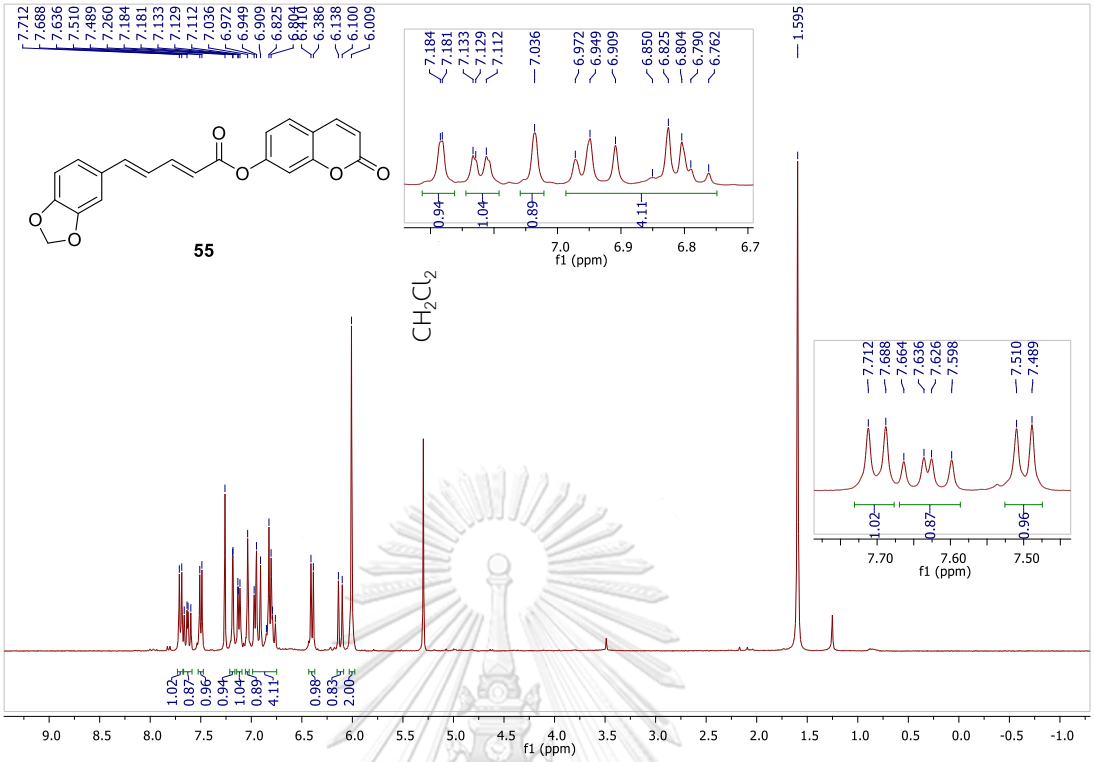


Figure A.59 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **55.**

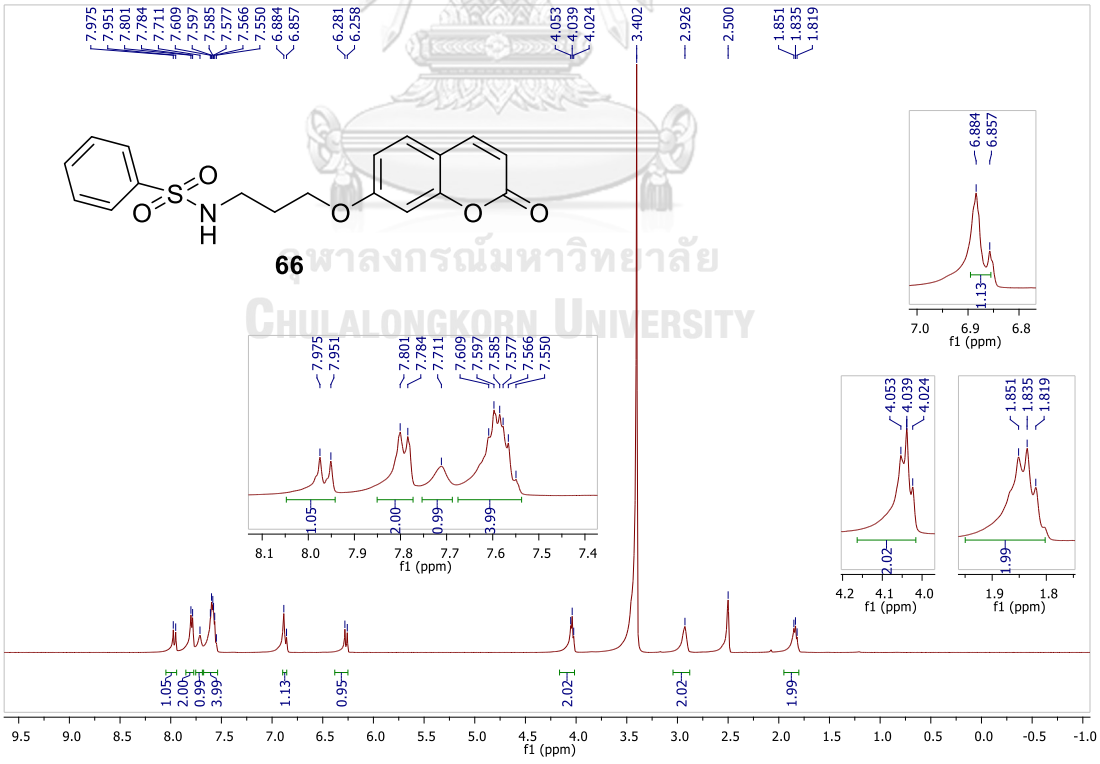


Figure A.60 ^{1}H-NMR (DMSO-d_6, 400 MHz) of **66.**

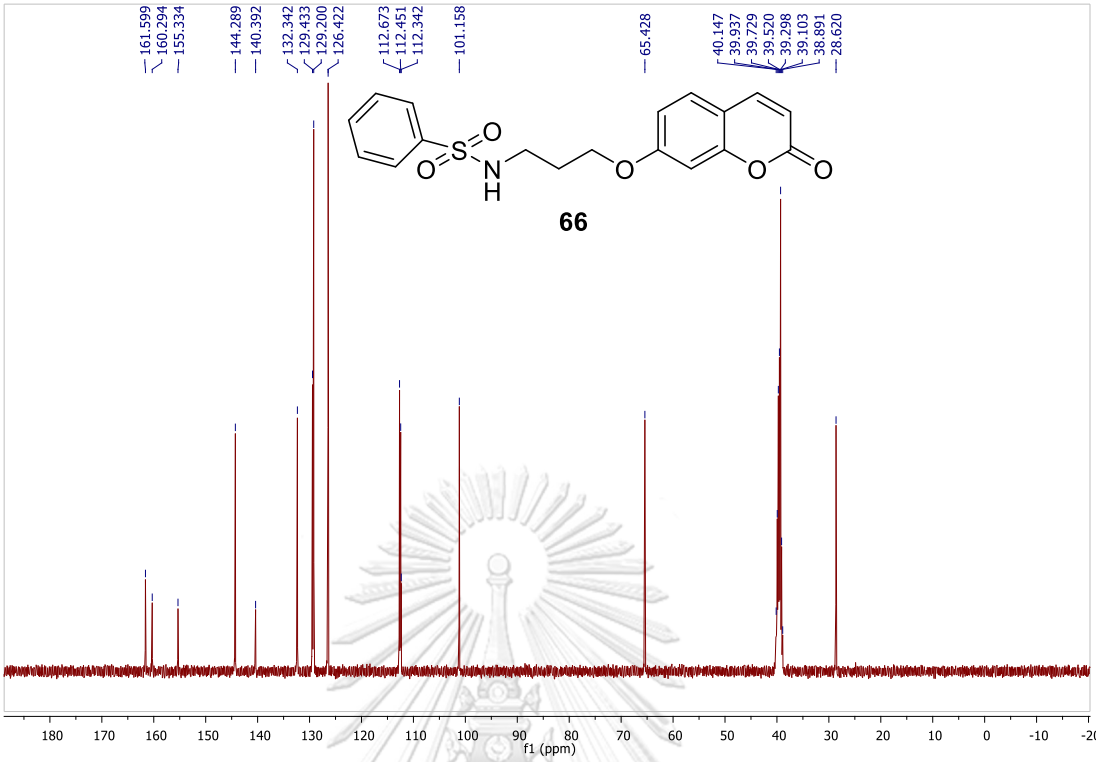


Figure A.61 ^{13}C-NMR (DMSO-d_6, 100 MHz) of **66.**

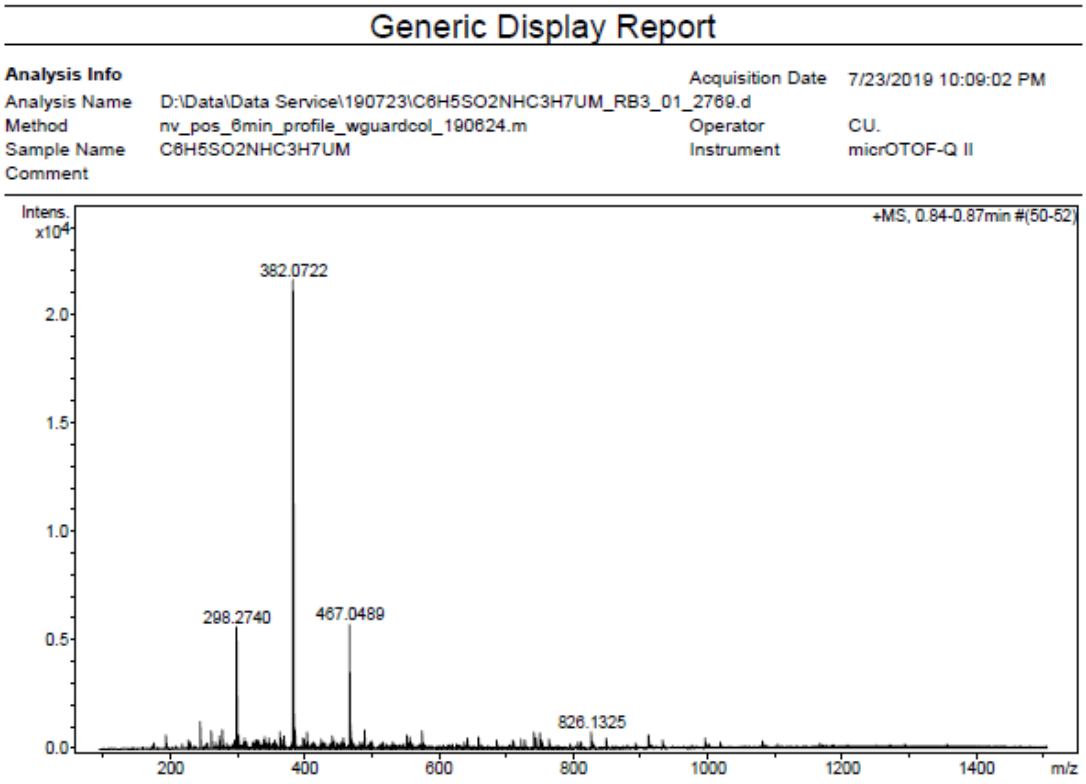


Figure A.62 HRMS of **66.**

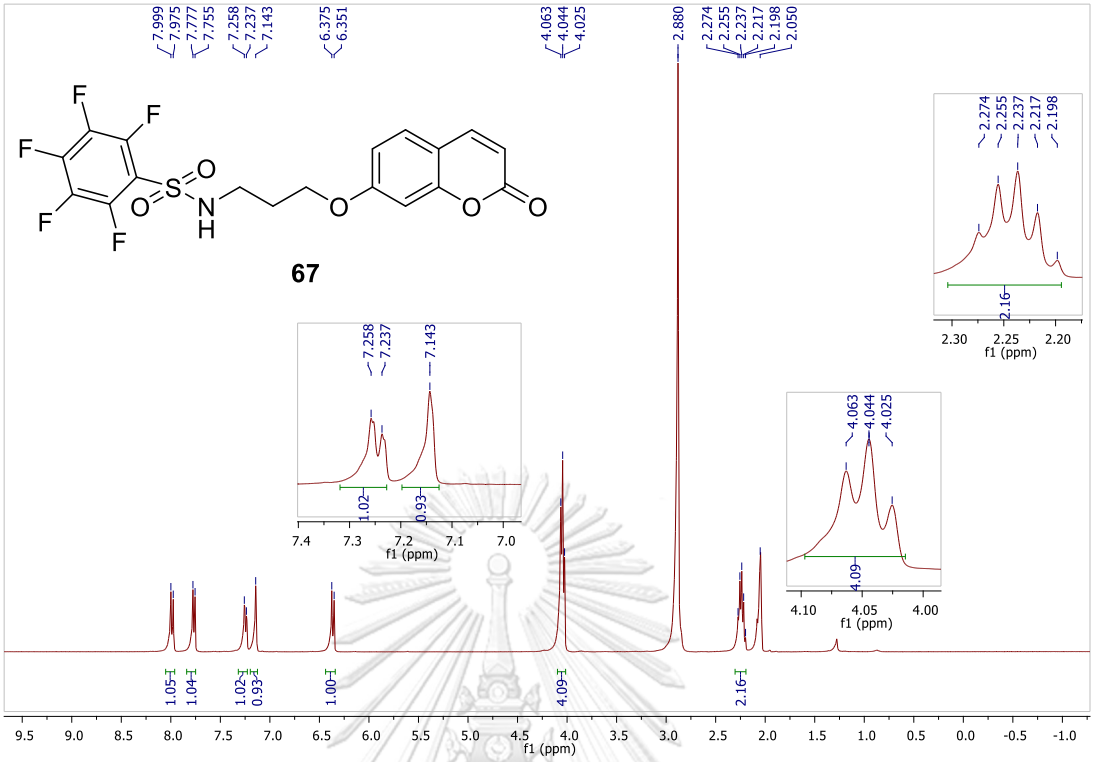


Figure A.63 ^{1}H-NMR (Acetone-d_6, 400 MHz) of **67.**

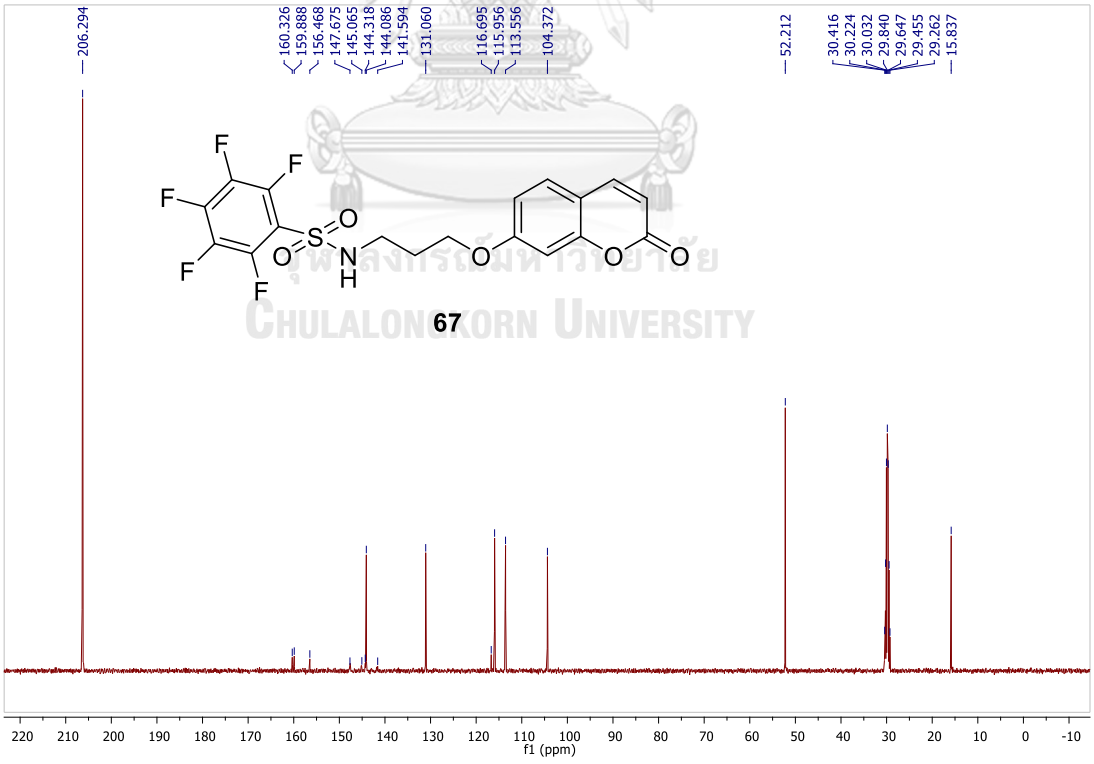


Figure A.64 ^{13}C-NMR (Acetone-d_6, 100 MHz) of **67.**

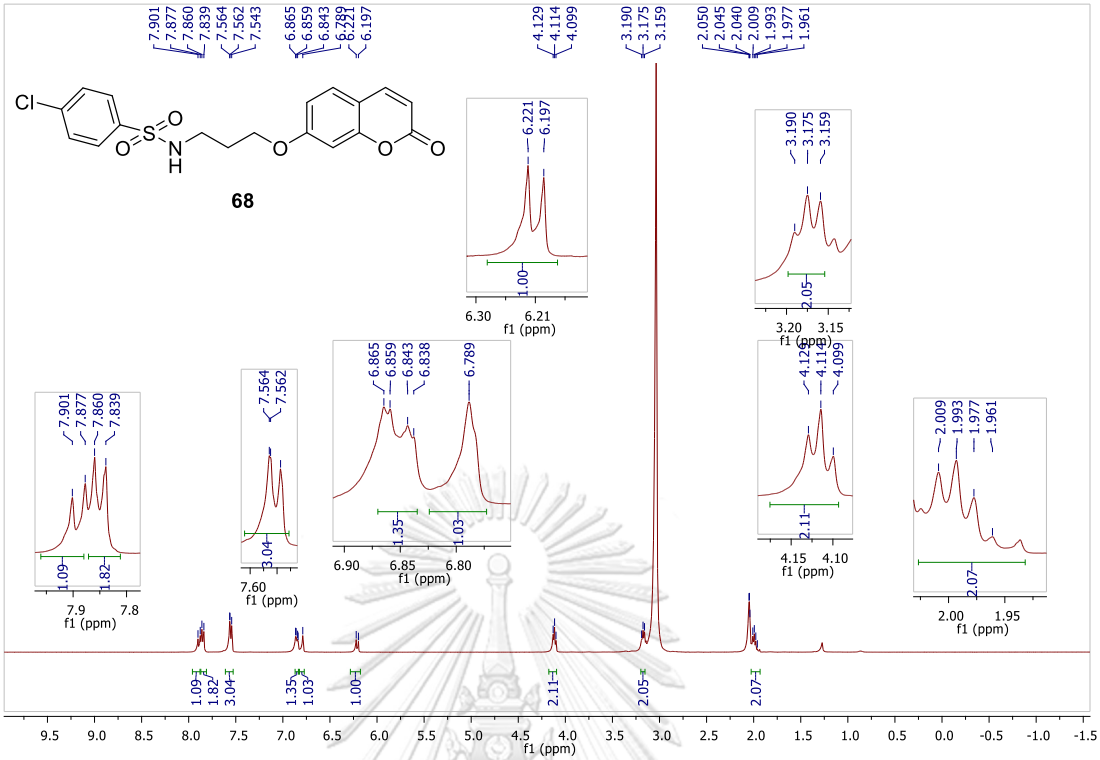


Figure A.65 ^{1}H-NMR (Acetone-d_6, 400 MHz) of **68**.

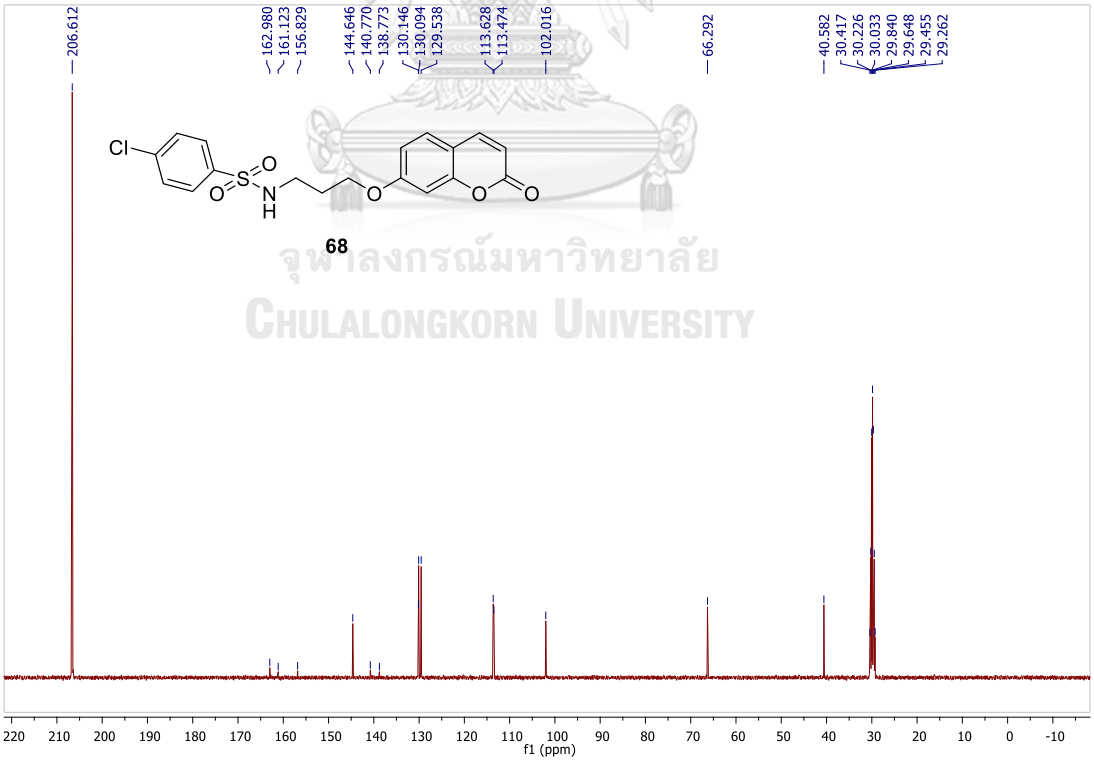


Figure A.66 ^{13}C-NMR (Acetone-d_6, 100 MHz) of **68**.

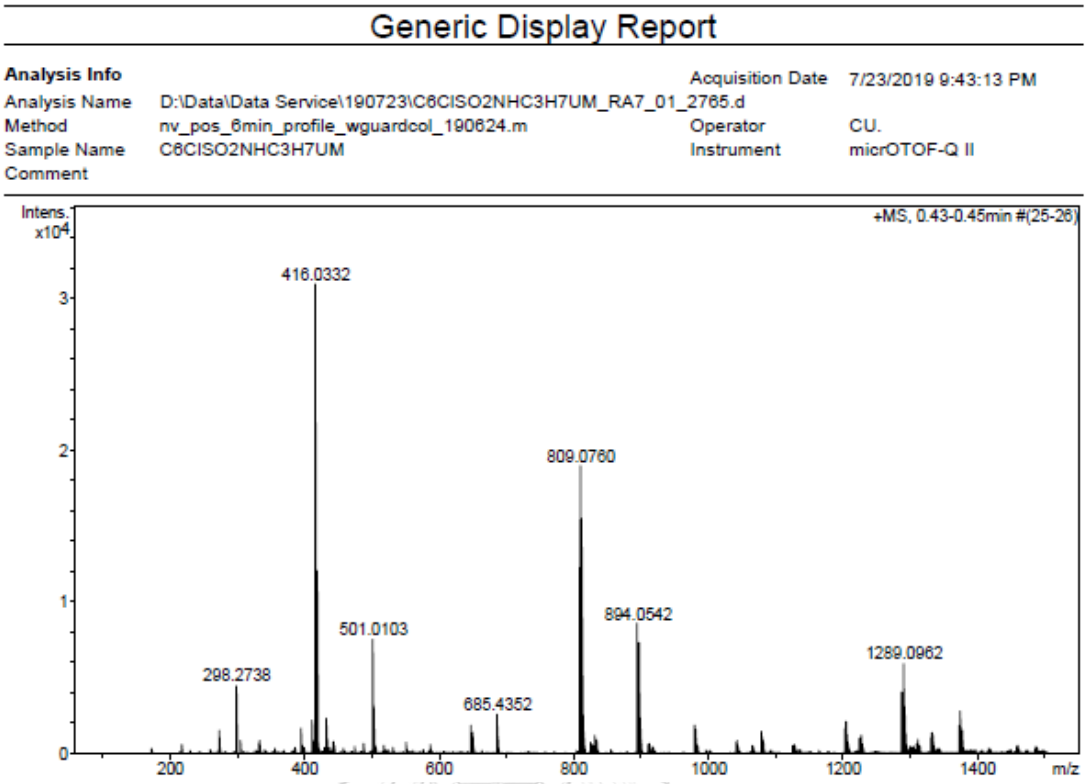


Figure A.67 HRMS of **68**.

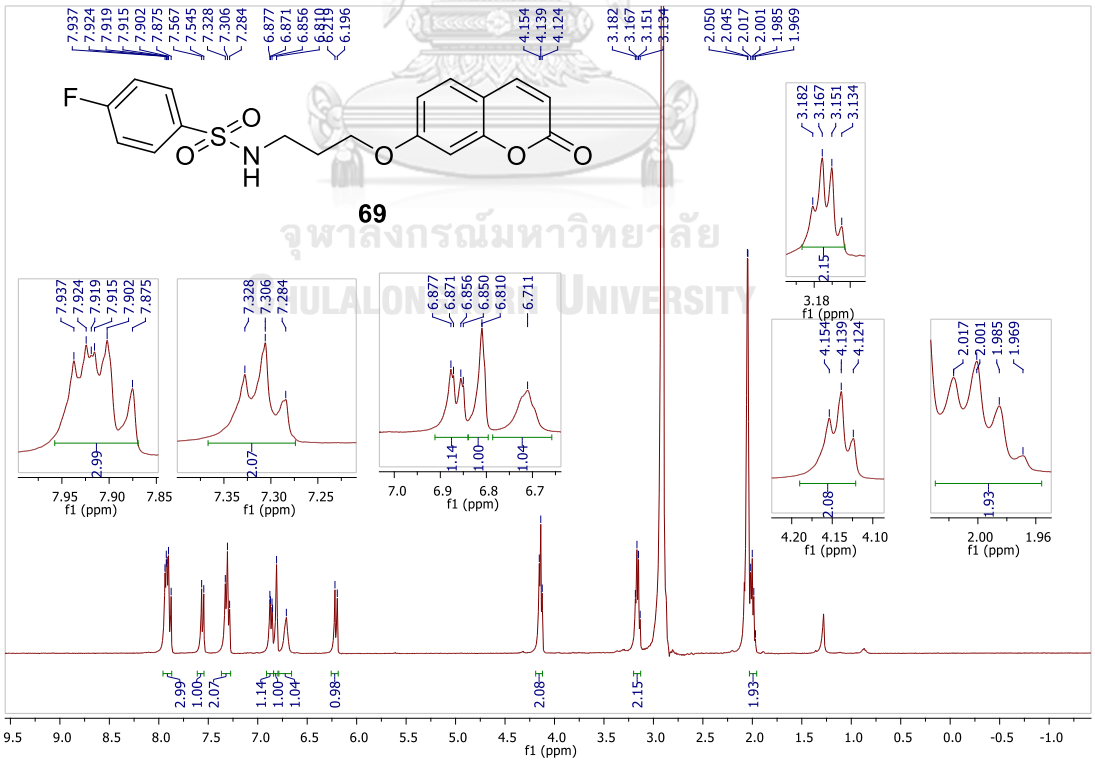


Figure A.68 ^{1}H-NMR (Acetone-d_6, 400 MHz) of **69**.

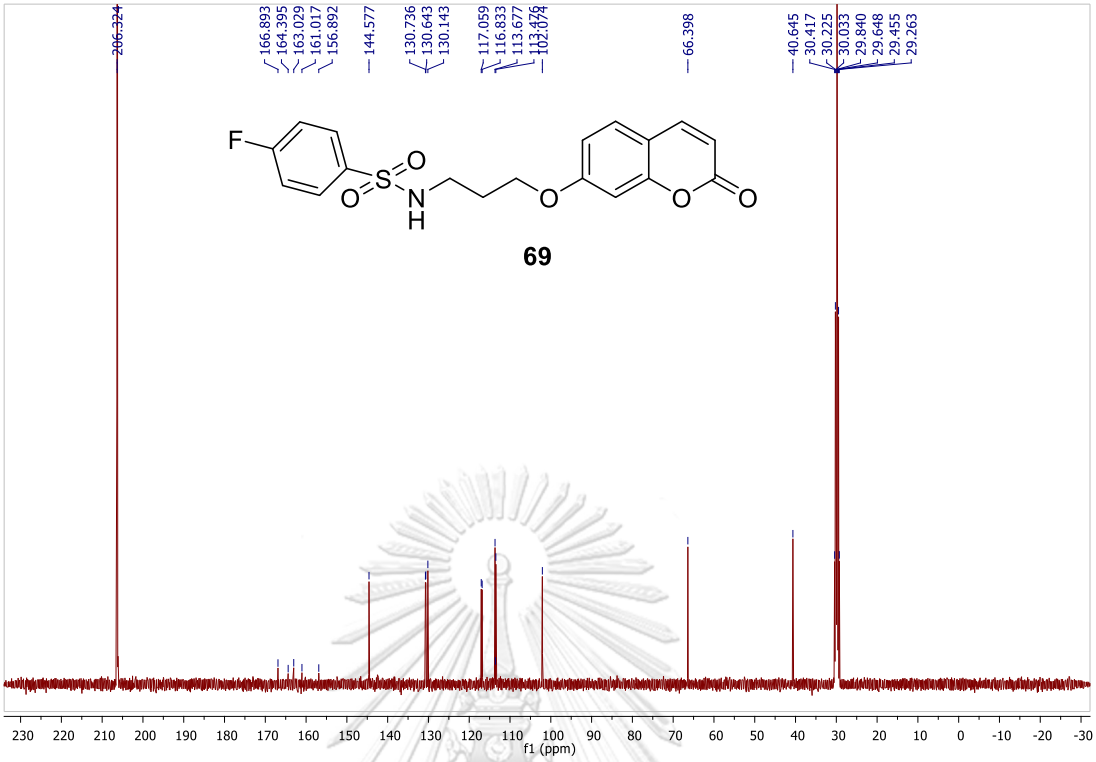


Figure A.69 ^{13}C-NMR (Acetone-d_6, 100 MHz) of **69.**

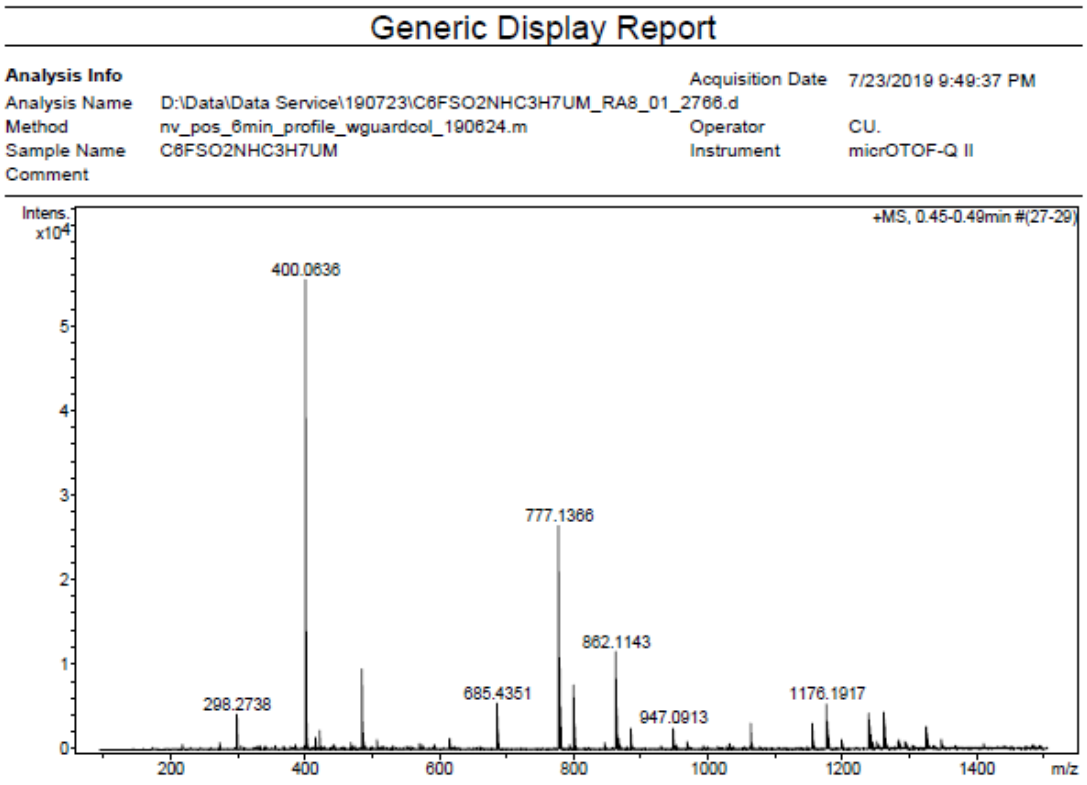


Figure A.70 HRMS of **69.**

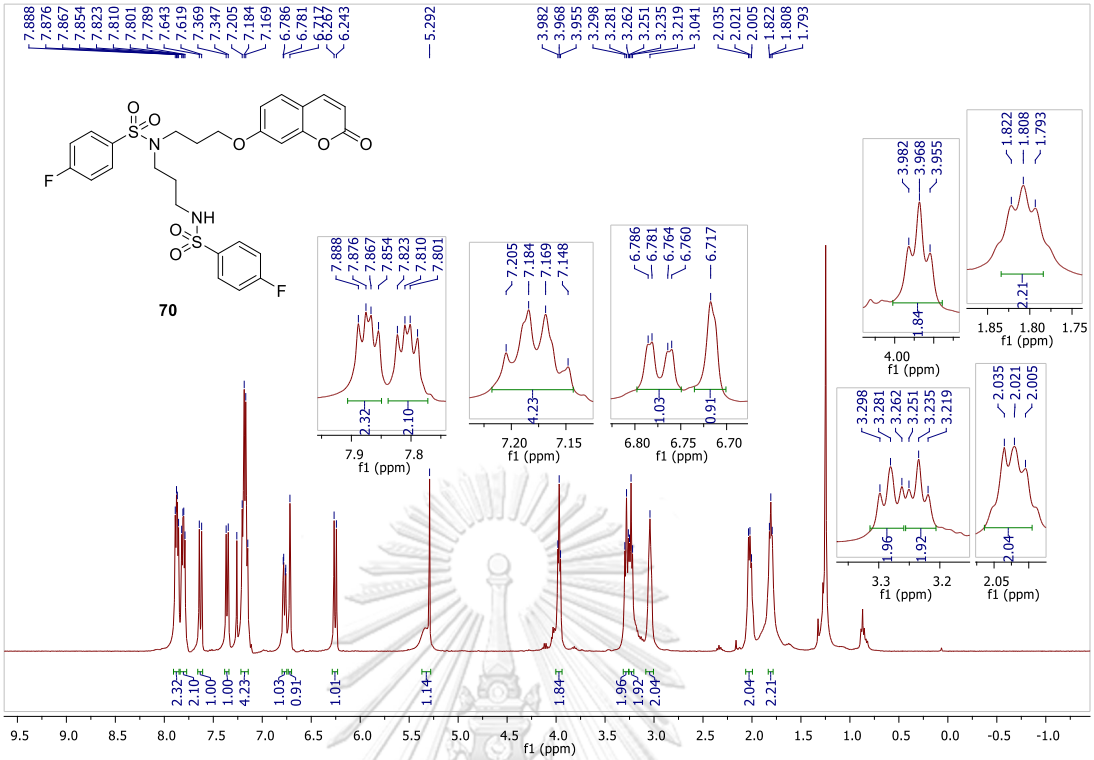


Figure A.71 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **70.**

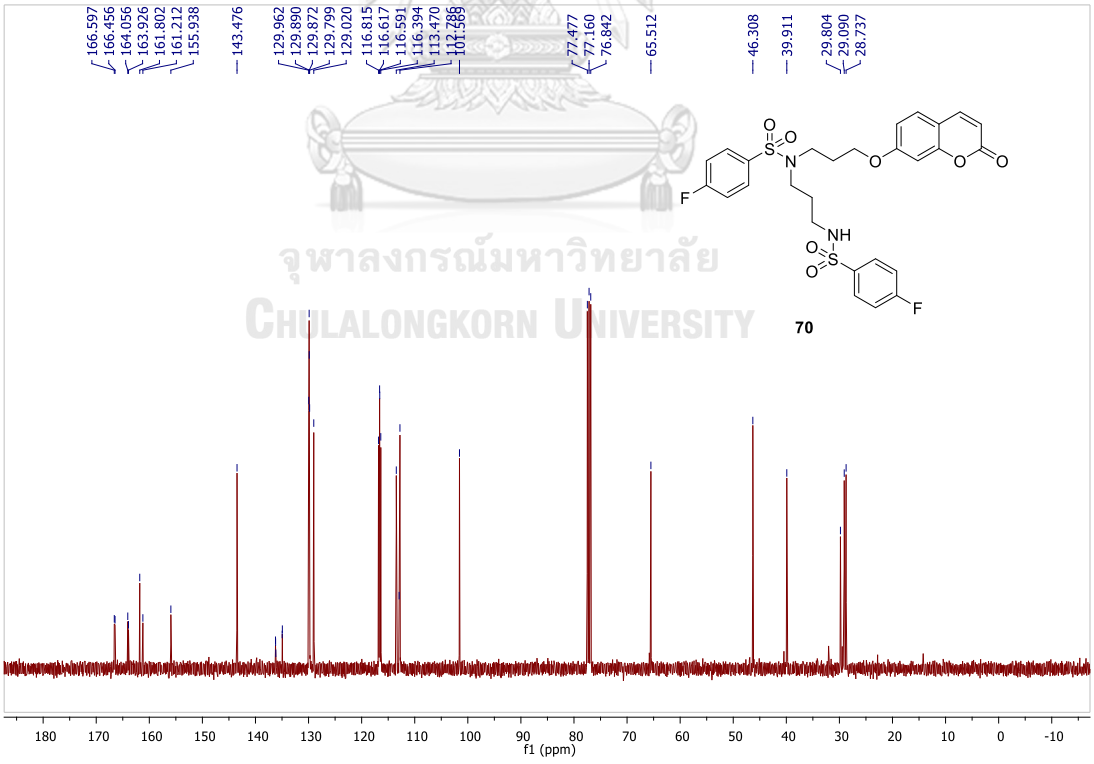


Figure A.72 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **70.**

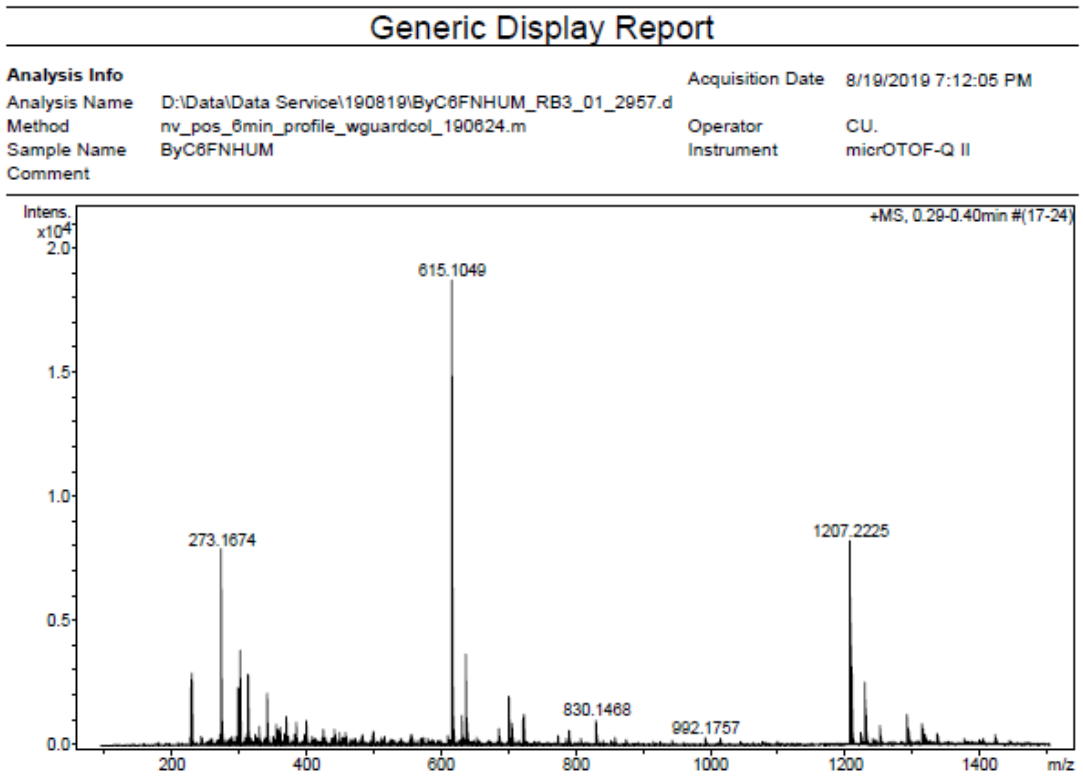


Figure A.73 HRMS of **70.**

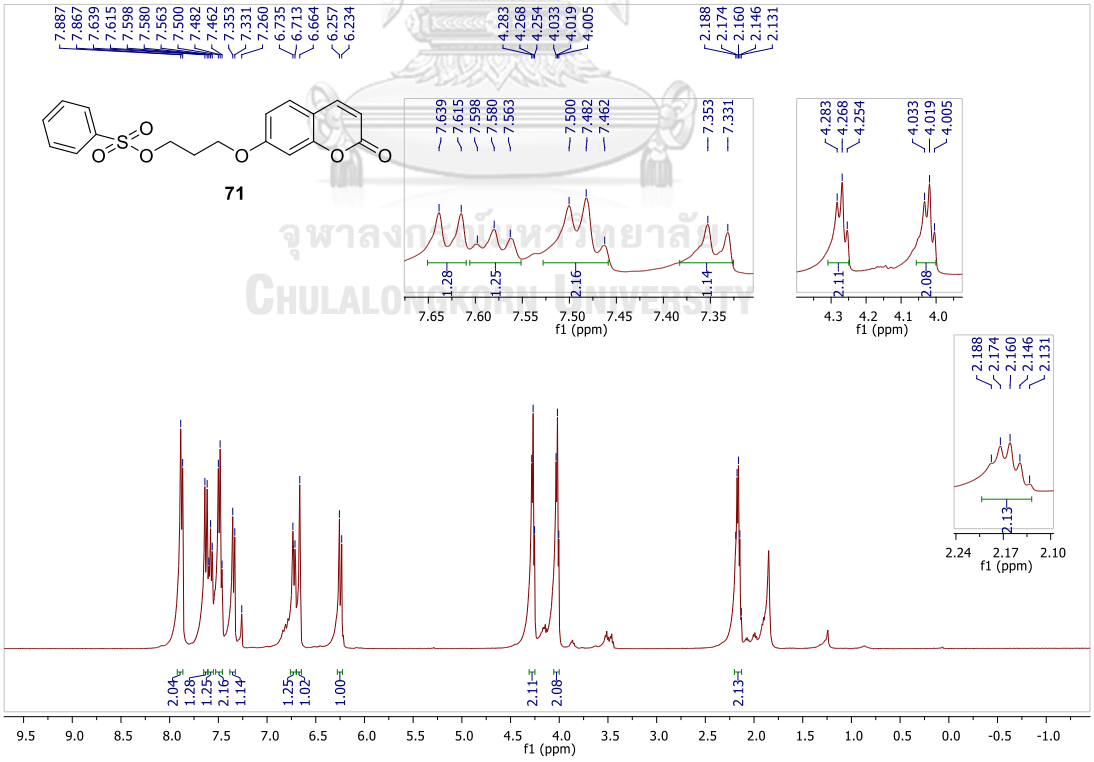


Figure A.74 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **71.**

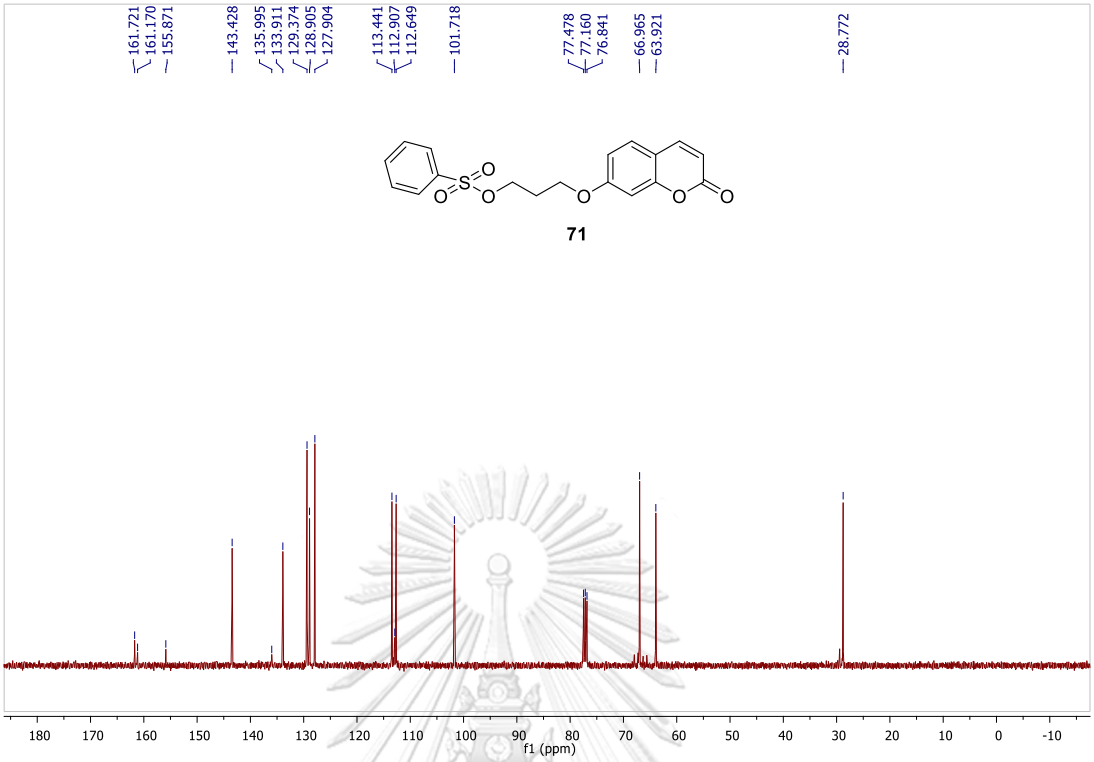


Figure A.75 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **71.**

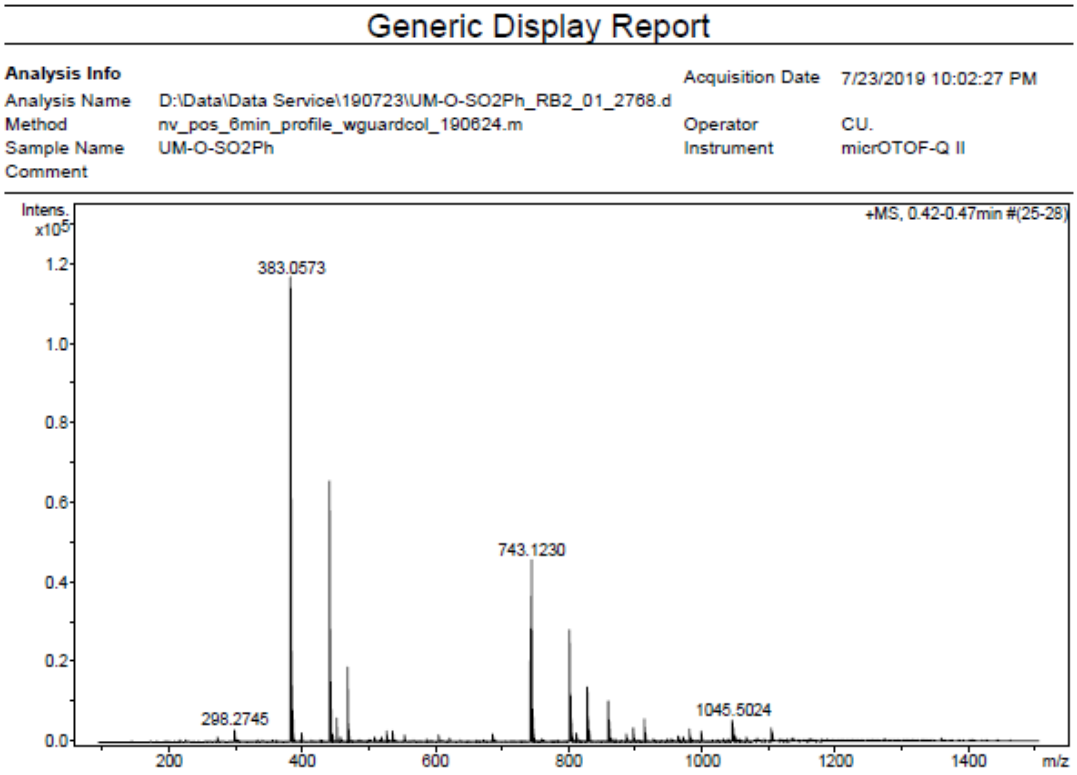


Figure A.76 HRMS of **71.**

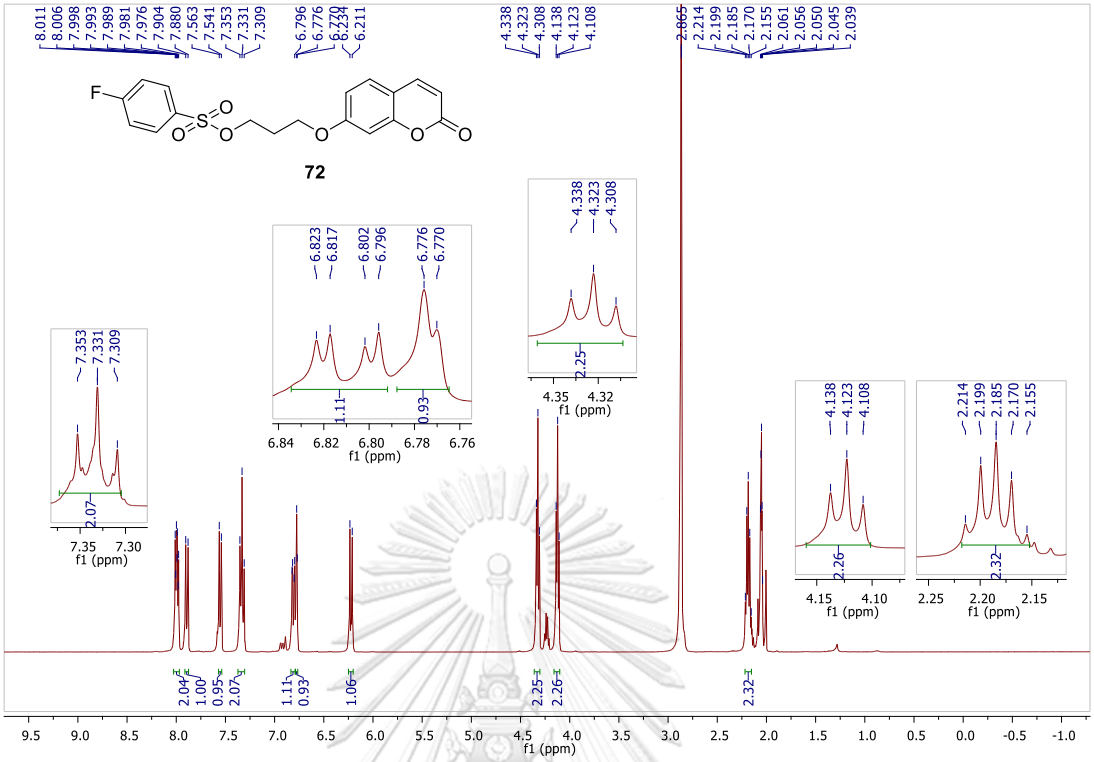


Figure A.77 ^{1}H-NMR (Acetone-d_6, 400 MHz) of **72.**

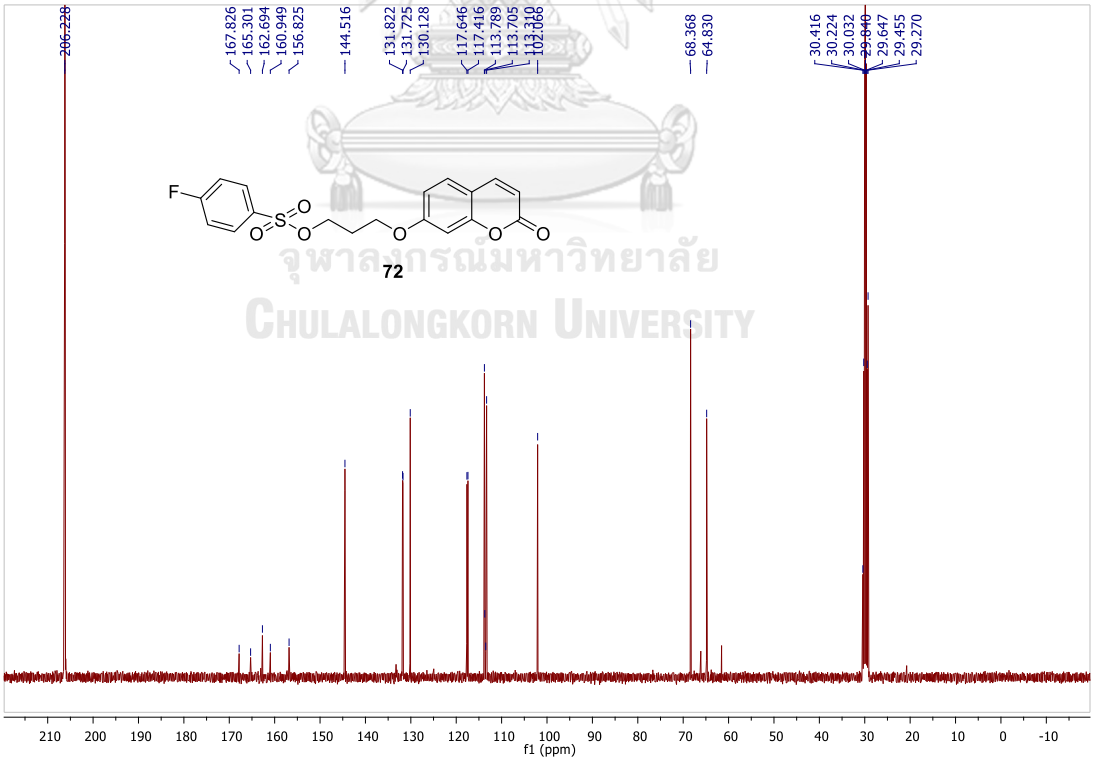


Figure A.78 ^{13}C-NMR (Acetone-d_6, 100 MHz) of **72**.

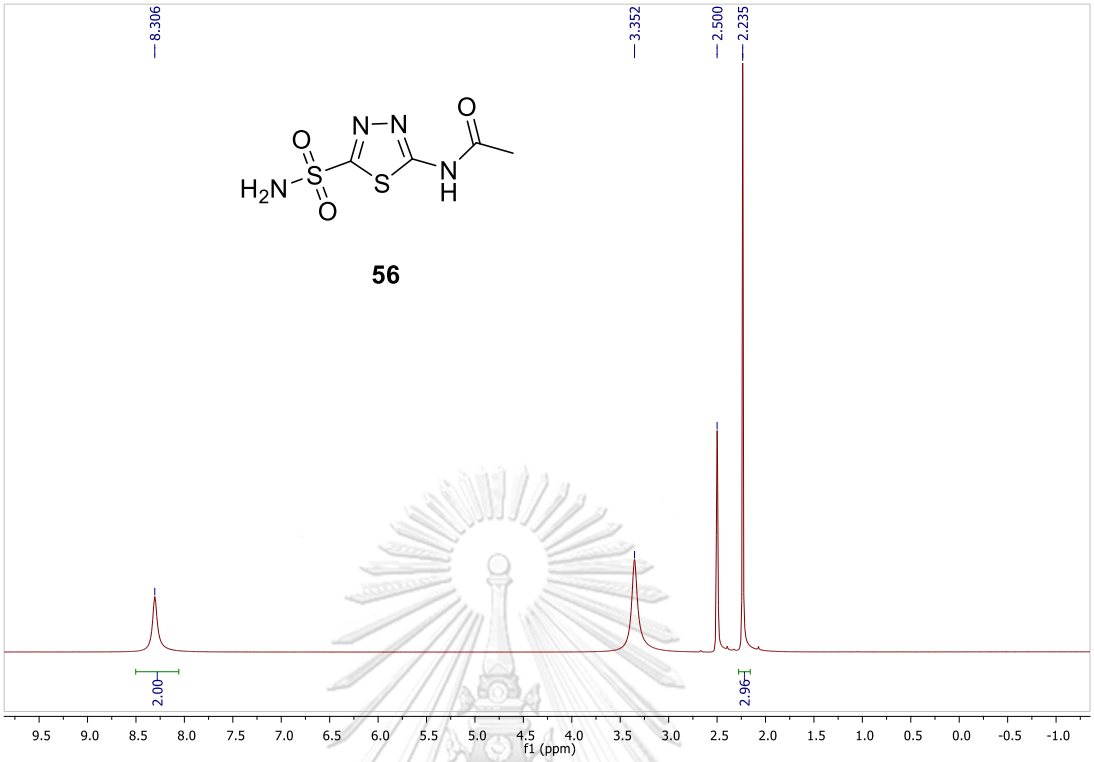


Figure A.79 ^{1}H-NMR (DMSO-d_6, 400 MHz) of **56**.

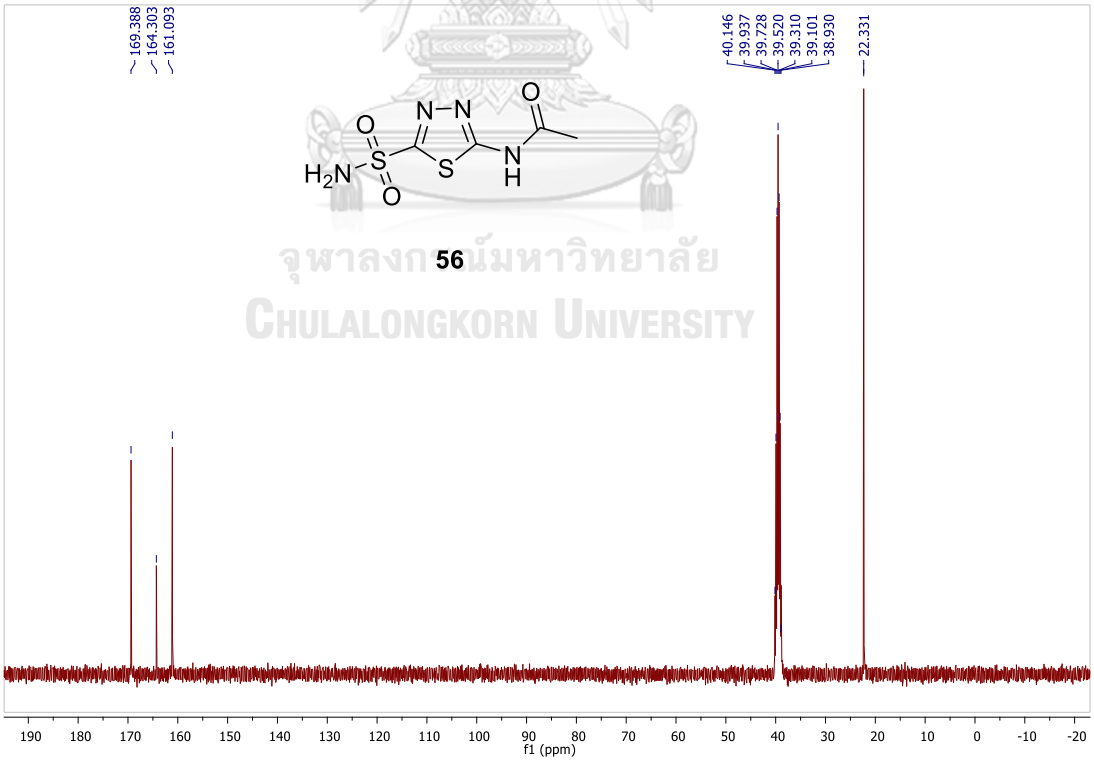


Figure A.80 ^{13}C-NMR (DMSO-d_6, 100 MHz) of **56**.

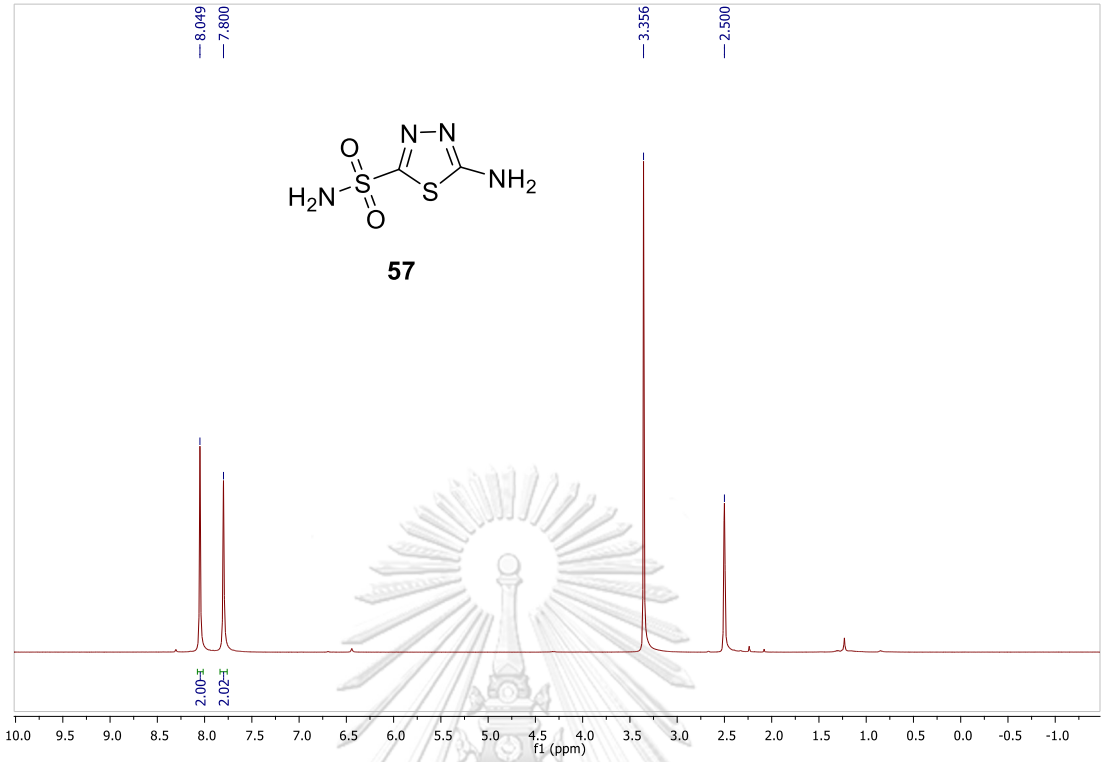


Figure A.81 ^{1}H-NMR (DMSO-d_6, 400 MHz) of **57.**

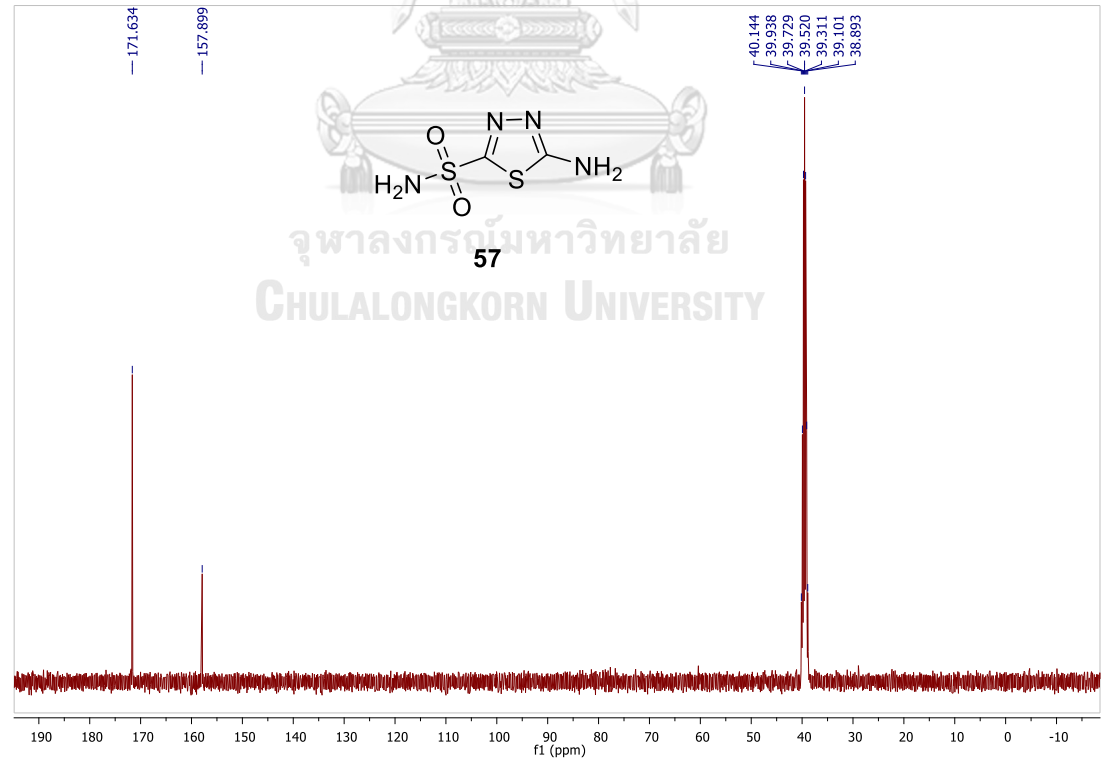


Figure A.82 ^{13}C-NMR (DMSO-d_6, 100 MHz) of **57**.

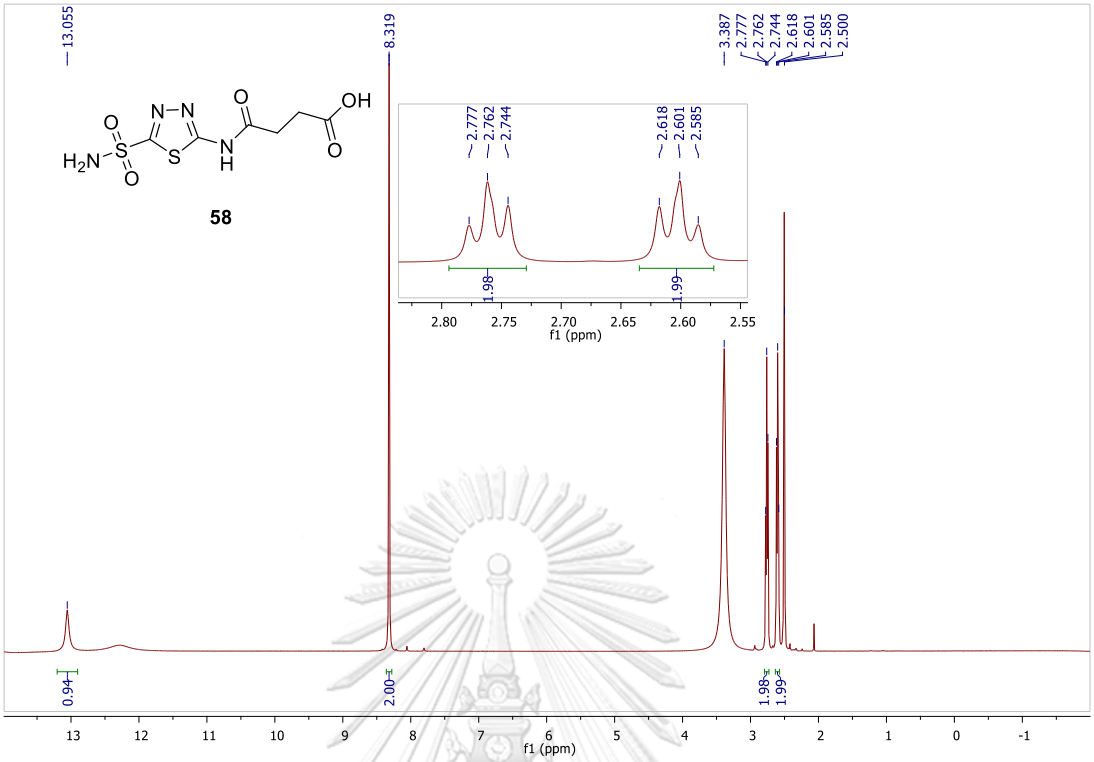


Figure A.83 ^{1}H-NMR (DMSO-d_6, 400 MHz) of **58.**

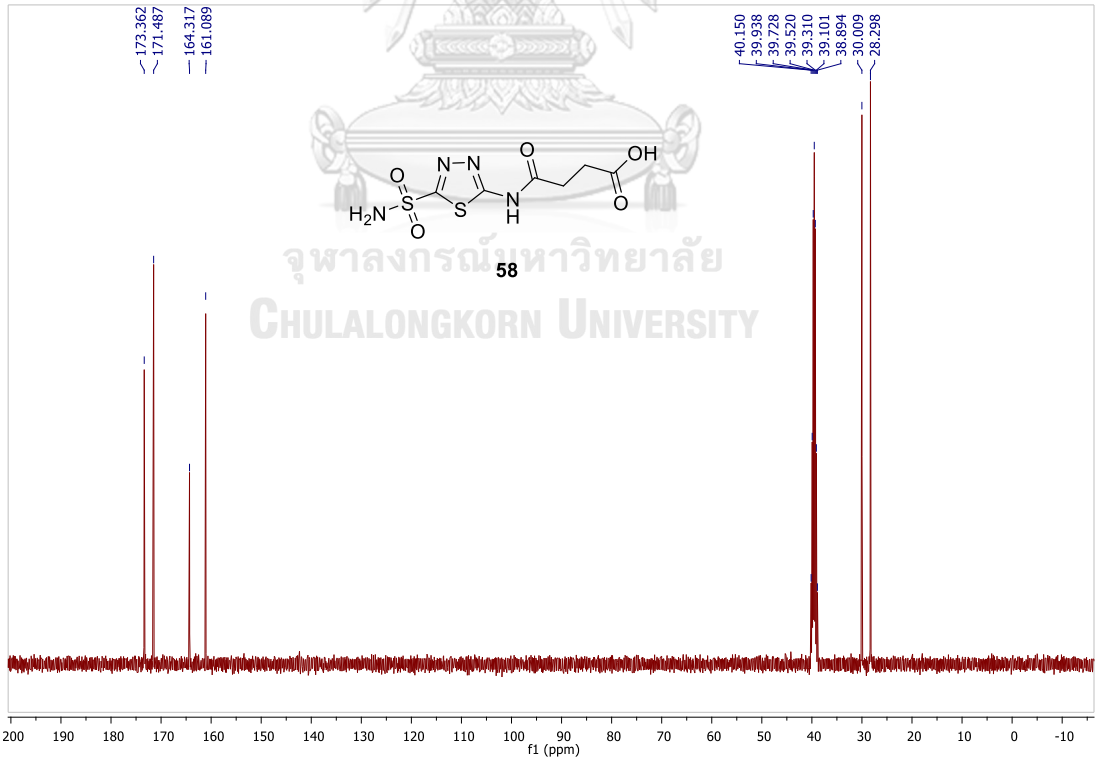


Figure A.84 ^{13}C-NMR (DMSO-d_6, 100 MHz) of **58**.

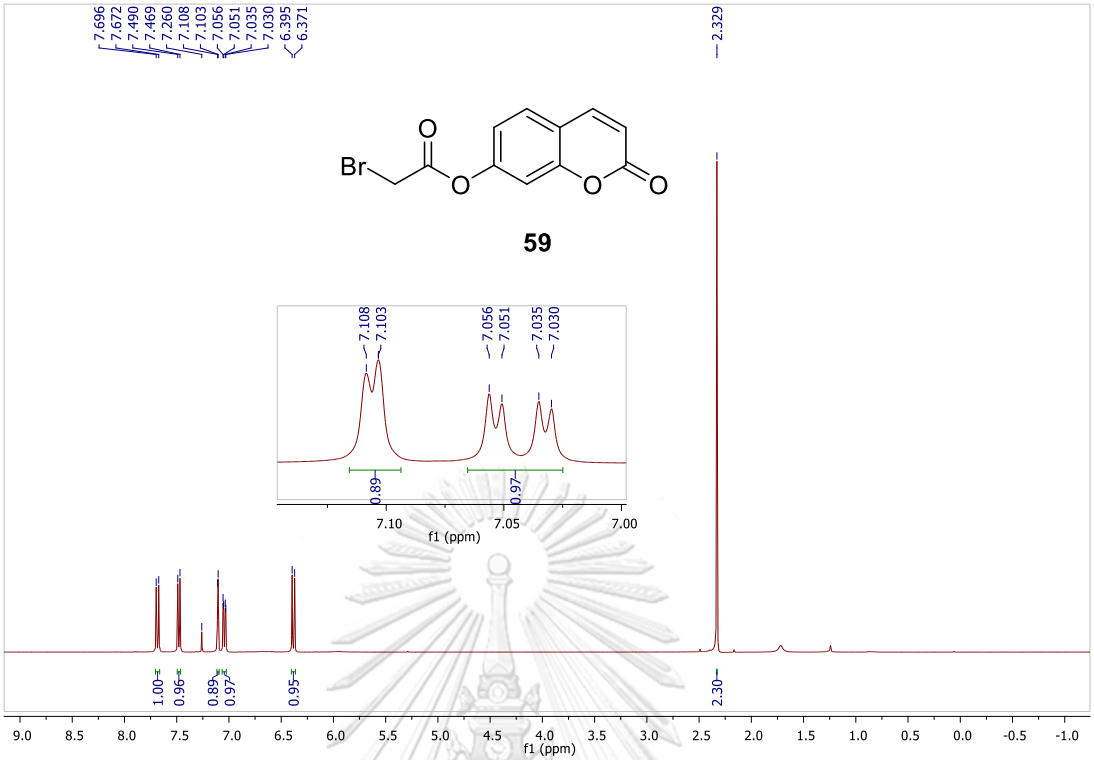


Figure A.85 ^{1}H-NMR ($CDCl_3$, 400 MHz) of **59.**

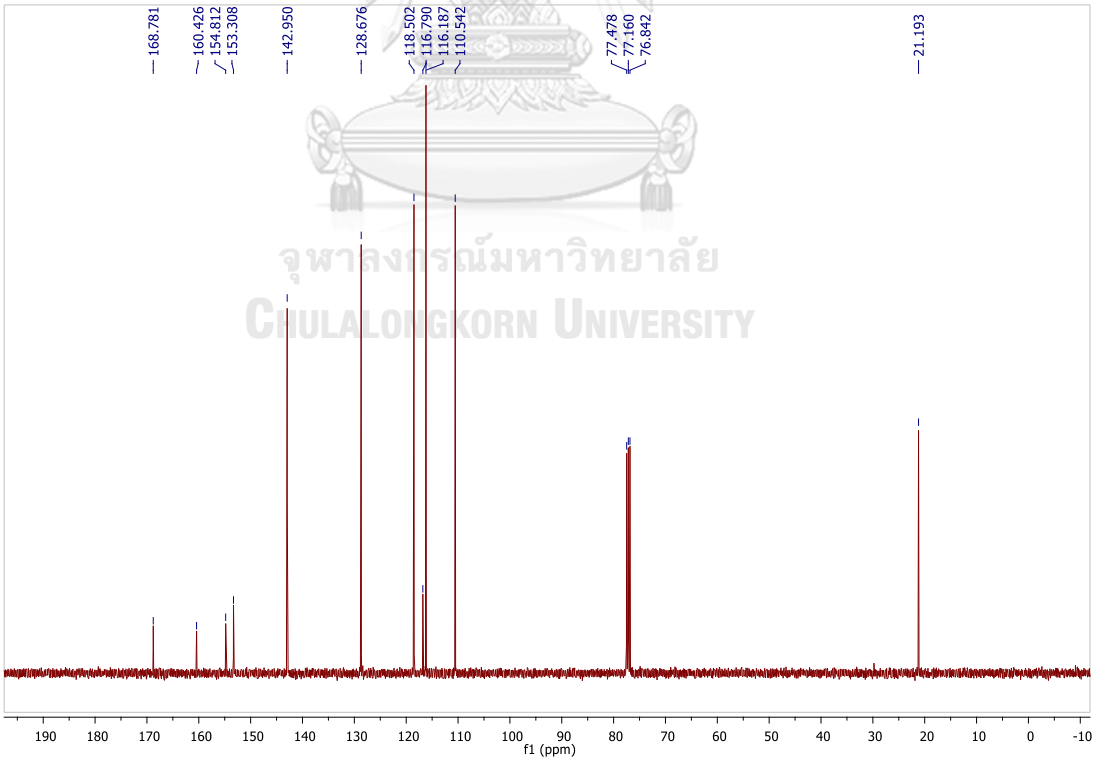


Figure A.86 ^{13}C-NMR ($CDCl_3$, 100 MHz) of **59.**

REFERENCES

1. De Luca, L.; Mancuso, F.; Ferro, S.; Buemi, M. R.; Angeli, A.; Del Prete, S.; Capasso, C.; Supuran, C. T.; Gitto, R., Inhibitory effects and structural insights for a novel series of coumarin-based compounds that selectively target human CA IX and CA XII carbonic anhydrases. *European journal of medicinal chemistry* **2018,** *143*, 276-282.

2. Ji, Q.; Ge, Z.; Ge, Z.; Chen, K.; Wu, H.; Liu, X.; Huang, Y.; Yuan, L.; Yang, X.; Liao, F., Synthesis and biological evaluation of novel phosphoramidate derivatives of coumarin as chitin synthase inhibitors and antifungal agents. *European journal of medicinal chemistry* **2016,** *108*, 166-176.

3. Ueberschaar, N.; Xu, Z.; Scherlach, K.; Metsä-Ketelä, M.; Bretschneider, T.; Dahse, H.-M.; Görls, H.; Hertweck, C., Synthetic remodeling of the chartreusin pathway to tune antiproliferative and antibacterial activities. *Journal of the American chemical society* **2013,** *135* (46), 17408-17416.

4. Liu, M.-M.; Chen, X.-Y.; Huang, Y.-Q.; Feng, P.; Guo, Y.-L.; Yang, G.; Chen, Y., Hybrids of phenylsulfonylfuroxan and coumarin as potent antitumor agents. *Journal of medicinal chemistry* **2014,** *57* (22), 9343-9356.

5. Amin, K. M.; Abou-Seri, S. M.; Awadallah, F. M.; Eissa, A. A.; Hassan, G. S.; Abdulla, M. M., Synthesis and anticancer activity of some 8-substituted-7-methoxy-2H-chromen-2-one derivatives toward hepatocellular carcinoma HepG2 cells. *European journal of medicinal chemistry* **2015,** *90*, 221-231.

6. Tsay, S.-C.; Hwu, J. R.; Singha, R.; Huang, W.-C.; Chang, Y. H.; Hsu, M.-H.; Shieh, F.-k.; Lin, C.-C.; Hwang, K. C.; Horng, J.-C., Coumarins hinged directly on benzimidazoles and their ribofuranosides to inhibit hepatitis C virus. *European journal of medicinal chemistry* **2013,** *63*, 290-298.

7. Kamiyama, H.; Kubo, Y.; Sato, H.; Yamamoto, N.; Fukuda, T.; Ishibashi, F.; Iwao, M., Synthesis, structure–activity relationships, and mechanism of action of anti-HIV-1 lamellarin α 20-sulfate analogues. *Bioorganic & medicinal chemistry* **2011,** *19* (24), 7541-7550.

8. Shaik, J. B.; Palaka, B. K.; Penumala, M.; Kotapati, K. V.; Devineni, S. R.;

Eadlapalli, S.; Darla, M. M.; Ampasala, D. R.; Vadde, R.; Amooru, G. D., Synthesis, pharmacological assessment, molecular modeling and in silico studies of fused tricyclic coumarin derivatives as a new family of multifunctional anti-Alzheimer agents. *European journal of medicinal chemistry* **2016,** *107*, 219-232.

9. Sashidhara, K. V.; Kumar, A.; Dodda, R. P.; Krishna, N. N.; Agarwal, P.; Srivastava, K.; Puri, S., Coumarin–trioxane hybrids: Synthesis and evaluation as a new class of antimalarial scaffolds. *Bioorganic & medicinal chemistry letters* **2012,** *22* (12), 3926-3930.

10. Pingaew, R.; Saekee, A.; Mandi, P.; Nantasenamat, C.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V., Synthesis, biological evaluation and molecular docking of novel chalcone–coumarin hybrids as anticancer and antimalarial agents. *European journal of medicinal chemistry* **2014,** *85*, 65-76.

11. Hu, Y.; Shen, Y.; Wu, X.; Tu, X.; Wang, G.-X., Synthesis and biological evaluation of coumarin derivatives containing imidazole skeleton as potential antibacterial agents. *European journal of medicinal chemistry* **2018,** *143*, 958-969.

12. Puttaraju, K. B.; Shivashankar, K.; Mahendra, M.; Rasal, V. P.; Vivek, P. N. V.; Rai, K.; Chanu, M. B., Microwave assisted synthesis of dihydrobenzo [4, 5] imidazo [1, 2-a] pyrimidin-4-ones; synthesis, in vitro antimicrobial and anticancer activities of novel coumarin substituted dihydrobenzo [4, 5] imidazo [1, 2-a] pyrimidin-4-ones. *European journal of medicinal chemistry* **2013,** *69*, 316-322.

13. Nagamallu, R.; Srinivasan, B.; Ningappa, M. B.; Kariyappa, A. K., Synthesis of novel coumarin appended bis (formylpyrazole) derivatives: Studies on their antimicrobial and antioxidant activities. *Bioorganic & medicinal chemistry letters* **2016,** *26* (2), 690-694.

14. Amin, K. M.; Rahman, D. E. A.; Al-Eryani, Y. A., Synthesis and preliminary evaluation of some substituted coumarins as anticonvulsant agents. *Bioorganic & medicinal chemistry* **2008,** *16* (10), 5377-5388.

15. Aggarwal, R.; Kumar, S.; Kaushik, P.; Kaushik, D.; Gupta, G. K., Synthesis and pharmacological evaluation of some novel 2-(5-hydroxy-5-trifluoromethyl-4, 5-dihydropyrazol-1-yl)-4-(coumarin-3-yl) thiazoles. *European journal of medicinal chemistry* **2013,** *62*, 508-514.

16. Ibrar, A.; Shehzadi, S. A.; Saeed, F.; Khan, I., Developing hybrid molecule therapeutics for diverse enzyme inhibitory action: Active role of coumarin-based structural leads in drug discovery. *Bioorganic & medicinal chemistry* **2018,** *26* (13), 3731-3762.

17. Hu, Y.-Q.; Xu, Z.; Zhang, S.; Wu, X.; Ding, J.-W.; Lv, Z.-S.; Feng, L.-S., Recent developments of coumarin-containing derivatives and their anti-tubercular activity. *European journal of medicinal chemistry* **2017,** *136*, 122-130.

18. Penta, S., *Advances in Structure and Activity Relationship of Coumarin Derivatives*. Academic Press: 2015.

19. Torres, F. C.; Brucker, N.; Fernandes Andrade, S.; Fabio Kawano, D.; Cristina Garcia, S.; Lino von Poser, G.; Lucia Eifler-Lima, V., New insights into the chemistry and antioxidant activity of coumarins. *Current topics in medicinal chemistry* **2014,** *14* (22), 2600-2623.

20. Supuran, C. T.; Scozzafava, A.; Casini, A., Carbonic anhydrase inhibitors. *Medicinal Research Reviews* **2003,** *23* (2), 146-189.

21. Supuran, C. T., Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature reviews Drug discovery* **2008,** *7* (2), 168-181.

22. Supuran, C. T., Carbonic anhydrase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2010,** *20* (12), 3467-3474.

23. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G., Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chemical reviews* **2012,** *112* (8), 4421-4468.

24. Frost, S. C.; McKenna, R., *Carbonic anhydrase: mechanism, regulation, links to disease, and industrial applications*. Springer Science & Business Media: 2013; Vol. 75.

25. Hassan, M. I.; Shajee, B.; Waheed, A.; Ahmad, F.; Sly, W. S., Structure, function and applications of carbonic anhydrase isozymes. *Bioorganic & medicinal chemistry* **2013,** *21* (6), 1570-1582.

26. Aggarwal, M.; Kondeti, B.; Tu, C.; Maupin, C. M.; Silverman, D. N.; McKenna, R., Structural insight into activity enhancement and inhibition of H64A carbonic anhydrase II by imidazoles. *IUCrJ* **2014,** *1* (2), 129-135.

27. Silverman, D. N.; Lindskog, S., The catalytic mechanism of carbonic anhydrase:

implications of a rate-limiting protolysis of water. *Accounts of Chemical Research* **1988,** *21* (1), 30-36.

28. Fisher, Z.; Hernandez Prada, J. A.; Tu, C.; Duda, D.; Yoshioka, C.; An, H.; Govindasamy, L.; Silverman, D. N.; McKenna, R., Structural and kinetic characterization of active-site histidine as a proton shuttle in catalysis by human carbonic anhydrase II. *Biochemistry* **2005,** *44* (4), 1097-1105.

29. Duda, D.; Tu, C.; Qian, M.; Laipis, P.; Agbandje-McKenna, M.; Silverman, D. N.; McKenna, R., Structural and kinetic analysis of the chemical rescue of the proton transfer function of carbonic anhydrase II. *Biochemistry* **2001,** *40* (6), 1741-1748.

30. Mantravadi, A.; Vadhar, N., Glaucoma. *Primary Care* **2015,** *42* (3), 437-449.

31. Quigley, H. A., Number of people with glaucoma worldwide. *British journal of ophthalmology* **1996,** *80* (5), 389-393.

32. Quigley, H. A.; Broman, A. T., The number of people with glaucoma worldwide in 2010 and 2020. *British journal of ophthalmology* **2006,** *90* (3), 262-267.

33. Schehlein, E. M.; Novack, G. D.; Robin, A. L., New classes of glaucoma medications. *Current opinion in ophthalmology* **2017,** *28* (2), 161-168.

34. Heijl, A.; Leske, M. C.; Bengtsson, B.; Hyman, L.; Bengtsson, B.; Hussein, M., Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Archives of ophthalmology* **2002,** *120* (10), 1268-1279.

35. Novack, G. D.; O'Donnell, M. J.; Molloy, D. W., New glaucoma medications in the geriatric population: efficacy and safety. *Journal of the American Geriatrics Society* **2002,** *50* (5), 956-962.

36. Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M., Carbonic anhydrase as a model for biophysical and physical-organic studies of proteins and protein– ligand binding. *Chemical reviews* **2008,** *108* (3), 946-1051.

37. Maresca, A.; Temperini, C.; Vu, H.; Pham, N. B.; Poulsen, S.-A.; Scozzafava, A.; Quinn, R. J.; Supuran, C. T., Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. *Journal of the American Chemical Society* **2009,** *131* (8), 3057-3062.

38. Maresca, A.; Temperini, C.; Pochet, L.; Masereel, B.; Scozzafava, A.; Supuran,

C. T., Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. *Journal of medicinal chemistry* **2010,** *53* (1), 335-344.

39. Vullo, D.; Franchi, M.; Gallori, E.; Antel, J.; Scozzafava, A.; Supuran, C. T., Carbonic anhydrase inhibitors. Inhibition of mitochondrial isozyme V with aromatic and heterocyclic sulfonamides. *Journal of medicinal chemistry* **2004,** *47* (5), 1272-1279.

40. Wang, Z.-C.; Qin, Y.-J.; Wang, P.-F.; Yang, Y.-A.; Wen, Q.; Zhang, X.; Qiu, H.-Y.; Duan, Y.-T.; Wang, Y.-T.; Sang, Y.-L., Sulfonamides containing coumarin moieties selectively and potently inhibit carbonic anhydrases II and IX: design, synthesis, inhibitory activity and 3D-QSAR analysis. *European journal of medicinal chemistry* **2013,** *66*, 1-11.

41. Kurt, B. Z.; Sönmez, F.; Bilen, Ç.; Ergun, A.; Gençer, N.; Arslan, O.; Kucukislamoglu, M., Synthesis, antioxidant and carbonic anhydrase I and II inhibitory activities of novel sulphonamide-substituted coumarylthiazole derivatives. *Journal of enzyme inhibition and medicinal chemistry* **2016,** *31* (6), 991-998.

42. Tanc, M.; Carta, F.; Bozdag, M.; Scozzafava, A.; Supuran, C. T., 7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors. *Bioorganic & medicinal chemistry* **2013,** *21* (15), 4502-4510.

43. Adfa, M.; Hattori, Y.; Yoshimura, T.; Koketsu, M., Antitermite activity of 7-alkoxycoumarins and related analogs against Coptotermes formosanus Shiraki. *International biodeterioration & biodegradation* **2012,** *74*, 129-135.

44. Bernini, R.; Pasqualetti, M.; Provenzano, G.; Tempesta, S., Ecofriendly synthesis of halogenated flavonoids and evaluation of their antifungal activity. *New journal of chemistry* **2015,** *39* (4), 2980-2987.

45. Sánchez-Recillas, A.; Navarrete-Vázquez, G.; Hidalgo-Figueroa, S.; Rios, M. Y.; Ibarra-Barajas, M.; Estrada-Soto, S., Semisynthesis, ex vivo evaluation, and SAR studies of coumarin derivatives as potential antiasthmatic drugs. *European journal of medicinal chemistry* **2014,** *77*, 400-408.

46. Kim, H. K.; Hairani, R.; Jeong, H.; Jeong, M. G.; Chavasiri, W.; Hwang, E. S., CBMG, a novel derivative of mansonone G suppresses adipocyte differentiation via suppression of PPAR**γ** activity. *Chemico-biological interactions* **2017,** *273*, 160-170.

47. More, K. N.; Lee, J. Y.; Kim, D.-Y.; Cho, N.-C.; Pyo, A.; Yun, M.; Kim, H. S.; Kim, H.; Ko, K.; Park, J.-H., Acetazolamide-based [18F]-PET tracer: In vivo validation of carbonic anhydrase IX as a sole target for imaging of CA-IX expressing hypoxic solid tumors. *Bioorganic & medicinal chemistry letters* **2018,** *28* (5), 915-921.

48. Akocak, S.; Alam, M. R.; Shabana, A. M.; Sanku, R. K. K.; Vullo, D.; Thompson, H.; Swenson, E. R.; Supuran, C. T.; Ilies, M. A., PEGylated bis-sulfonamide carbonic anhydrase inhibitors can efficiently control the growth of several carbonic anhydrase IX-expressing carcinomas. *Journal of medicinal chemistry* **2016,** *59* (10), 5077-5088.

49. Sangkaew, A.; Krungkrai, J.; Yompakdee, C., Development of a high throughput yeast-based screening assay for human carbonic anhydrase isozyme II inhibitors. *AMB Express* **2018,** *8* (1), 124.

50. Silva, F. S.; Starostina, I. G.; Ivanova, V. V.; Rizvanov, A. A.; Oliveira, P. J.; Pereira, S. P., Determination of metabolic viability and cell mass using a tandem resazurin/sulforhodamine B assay. *Current protocols in toxicology* **2016,** *68* (1), 2.24. 1-2.24. 15.

51. Zubía, E.; Luis, F. R.; Massanet, G. M.; Collado, I. G., An efficient synthesis of furanocoumarins. *Tetrahedron* **1992,** *48* (20), 4239-4246.

52. Carta, F.; Maresca, A.; Scozzafava, A.; Supuran, C. T., Novel coumarins and 2-thioxo-coumarins as inhibitors of the tumor-associated carbonic anhydrases IX and XII. *Bioorganic & medicinal chemistry* **2012,** *20* (7), 2266-2273.

53. Yang, X.; Wedajo, W.; Yamada, Y.; Dahlroth, S.-L.; Neo, J. J.-L.; Dick, T.; Chui, W.-K., 1, 3, 5-triazaspiro [5.5] undeca-2, 4-dienes as selective Mycobacterium tuberculosis dihydrofolate reductase inhibitors with potent whole cell activity. *European journal of medicinal chemistry* **2018,** *144*, 262-276.

54. Zhao, H.; Yan, B.; Peterson, L. B.; Blagg, B. S., 3-Arylcoumarin derivatives manifest anti-proliferative activity through Hsp90 inhibition. *ACS medicinal chemistry letters* **2012,** *3* (4), 327-331.

55. Stocker, P.; Cassien, M.; Vidal, N.; Thétiot-Laurent, S.; Pietri, S., A fluorescent homogeneous assay for myeloperoxidase measurement in biological samples. A positive correlation between myeloperoxidase-generated HOCl level and oxidative status in STZ-diabetic rats. *Talanta* **2017,** *170*, 119-127.

56. Supuran, C. T.; Scozzafava, A., Carbonic anhydrase inhibitors and their therapeutic potential. *Expert Opinion on Therapeutic Patents* **2000,** *10* (5), 575-600.

57. Riveiro, M. E.; De Kimpe, N.; Moglioni, A.; Vazquez, R.; Monczor, F.; Shayo, C.; Davio, C., Coumarins: old compounds with novel promising therapeutic perspectives. *Current medicinal chemistry* **2010,** *17* (13), 1325-1338.

58. Khan, K. M.; Rahim, F.; Wadood, A.; Kosar, N.; Taha, M.; Lalani, S.; Khan, A.; Fakhri, M. I.; Junaid, M.; Rehman, W., Synthesis and molecular docking studies of potent α-glucosidase inhibitors based on biscoumarin skeleton. *European journal of medicinal chemistry* **2014,** *81*, 245-252.

59. Zawawi, N. K. N. A.; Taha, M.; Ahmat, N.; Ismail, N. H.; Wadood, A.; Rahim, F.; Rehman, A. U., Synthesis, in vitro evaluation and molecular docking studies of biscoumarin thiourea as a new inhibitor of α-glucosidases. *Bioorganic chemistry* **2015,** *63*, 36-44.

60. Raju, B. C.; Tiwari, A. K.; Kumar, J. A.; Ali, A. Z.; Agawane, S. B.; Saidachary, G.; Madhusudana, K., α-Glucosidase inhibitory antihyperglycemic activity of substituted chromenone derivatives. *Bioorganic & medicinal chemistry* **2010,** *18* (1), 358-365.

61. Shen, Q.; Shao, J.; Peng, Q.; Zhang, W.; Ma, L.; Chan, A. S.; Gu, L., Hydroxycoumarin derivatives: novel and potent α-glucosidase inhibitors. *Journal of medicinal chemistry* **2010,** *53* (23), 8252-8259.

62. Wang, S.; Yan, J.; Wang, X.; Yang, Z.; Lin, F.; Zhang, T., Synthesis and evaluation of the α-glucosidase inhibitory activity of 3-[4-(phenylsulfonamido) benzoyl]-2H-1-benzopyran-2-one derivatives. *European journal of medicinal chemistry* **2010,** *45* (3), 1250-1255.

63. Dove, A., Seeking sweet relief for diabetes. *Nature biotechnology* **2002,** *20* (10), 977.

64. Association, A. D., Diagnosis and classification of diabetes mellitus. *Diabetes care* **2010,** *33* (Supplement 1), S62-S69.

65. van de Laar, F. A., Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. *Vascular health and risk management* **2008,** *4* (6), 1189.

66. Nathan, D. M., Finding new treatments for diabetes—how many, how fast… how

good? *New England Journal of Medicine* **2007,** *356* (5), 437-440.

67. Newcomer, A. D.; McGill, D. B., Distribution of disaccharidase activity in the small bowel of normal and lactase-deficient subjects. *Gastroenterology* **1966,** *51*, 481-488.

68. Tan, M.-H., α-Glucosidase inhibitors in the treatment of diabetes. *Current Opinion in Endocrinology, Diabetes and Obesity* **1997,** *4* (1), 48-55.

69. Jacob, G. S., Glycosylation inhibitors in biology and medicine. *Current opinion in structural biology* **1995,** *5* (5), 605-611.

70. Balfour, J. A.; McTavish, D., Acarbose. *Drugs* **1993,** *46* (6), 1025-1054.

71. Bischoff, H., The mechanism of alpha-glucosidase inhibition in the management of diabetes. *Clinical and investigative medicine. Medecine clinique et experimentale* **1995,** *18* (4), 303-311.

72. Rosak, C.; Mertes, G., Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes, metabolic syndrome and obesity: targets and therapy* **2012,** *5*, 357.

73. DiNicolantonio, J. J.; Bhutani, J.; O'Keefe, J. H., Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes. *Open heart* **2015,** *2* (1), e000327.

74. Ramesh, B.; Pugalendi, K., Antihyperglycemic effect of umbelliferone in streptozotocin-diabetic rats. *Journal of medicinal food* **2006,** *9* (4), 562-566.

75. Bassetto, M.; Leyssen, P.; Neyts, J.; Yerukhimovich, M. M.; Frick, D. N.; Courtney-Smith, M.; Brancale, A., In silico identification, design and synthesis of novel piperazine-based antiviral agents targeting the hepatitis C virus helicase. *European journal of medicinal chemistry* **2017,** *125*, 1115-1131.

76. Tano; Nobu, Preparation of perfluoropolyether-linked tetraalkylammonium salt as ionic liquids and lubricants for magnetic recording medium and magnetic recording medium. *World Intellectual Property Organization* **2018**, 1-62.

77. Ramadhan, R.; Phuwapraisirisan, P., New arylalkanones from Horsfieldia macrobotrys, effective antidiabetic agents concomitantly inhibiting α-glucosidase and free radicals. *Bioorganic & medicinal chemistry letters* **2015,** *25* (20), 4529-4533.

78. Lien, E. J.; Hussain, M.; Tong, G. L., Role of hydrophobic interactions in enzyme

inhibition by drugs. *Journal of pharmaceutical sciences* **1970,** *59* (6), 865-868.

79. Hairani, R.; Mongkol, R.; Chavasiri, W., Allyl and prenyl ethers of mansonone G, new potential semisynthetic antibacterial agents. *Bioorganic & medicinal chemistry letters* **2016,** *26* (21), 5300-5303.

VITA

NAME	Truc Phan Thi Hong
DATE OF BIRTH	9 May 1995
PLACE OF BIRTH	Bao Loc city, Vietnam
INSTITUTIONS ATTENDED	Department of Chemistry, University of Education, HCMC, Vietnam
HOME ADDRESS	Room 219, Diana court, soi 6, Phetchaburi, Ratchathewi, Bangkok, Thailand