

SYNTHESIS AND BIOACTIVITY STUDIES OF COUMARIN AND ITS
DERIVATIVES

WONG KOK TONG

UNIVERSITI TEKNOLOGI MALAYSIA

SYNTHESIS AND BIOACTIVITY STUDIES OF COUMARIN AND ITS
DERIVATIVES

WONG KOK TONG

A thesis submitted in fulfilment of the
Requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

JULY 2013

Dedicated to my beloved family

ACKNOWLEDGEMENT

First and foremost, praise to the Buddha, Dhamma and Sangka.

I would like to extend my sincerest appreciation to my supervisor, Dr. Norazah binti Basar and Dr. Razauden bin Mohamed Zulkifli for their guidance, help, inspiration, attention, encouragement and advices to accomplish throughout my work. Not forgotten to Assoc. Prof. Dr. Farediah binti Ahmad for her invaluable assistance and continuous supports throughout this project.

My appreciation also goes to all the academic staff of Chemistry Department, all laboratory staff (Mdm. Ain, Mr. Azmi, Mr. Rasyidi and Mdm. Nasrena), and fellow friends (Athirah, Amira, Atikah, Atiqah, Awanis, Kiew Siaw Fui, Mariam, Noraimi and others) willing to lend a helping hand.

It is a pleasure to pay tribute also to Dr. Lisa Harris of University College London for the Mass Spectrum Analysis. I would also acknowledge Ministry of Higher Education Malaysia (MOHE) for giving me the scholarship to pursue my Master degree at Universiti Teknologi Malaysia.

Furthermore, my utmost grateful to my parents (Wong Soon See and Wong Yoke Hun) and my entire family (Wong Kok Wei, Chow Chui See, Wong Kok Yeong and Wong Pei Yee) for their encouragement and valuable supports. Last but not least, I would also to thank who are involved directly and indirectly in the completion of this study.

PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between July 2010 and August 2012 under the supervision of Dr. Norazah Basar and Dr Razauden bin Mohamed Zulkifli. Parts of my work described in this thesis have been reported in the following publications:

1. Kok Tong Wong and Norazah Basar (2012). Coumarins *via* Knoevenagel Condensation Reaction (KCR) and Pechmann Condensation Reaction. National Science Postgraduate Conference 2011 (NSPC), 16-17 November 2011, at Ibnu Sina Institute, UTM, Johor Bahru, Malaysia. *Jurnal Teknologi*. **57**: 83-98.
2. Kok Tong Wong, Norazah Basar and Razauden Mohamed Zulkifli (2012). Synthesis of Substituted Coumarin. Paper presented at the 2nd National Symposium In Organic Synthesis 2012 (NaSOS II) at Concorde Hotel, Shah Alam, Selangor. 16-17 July 2012.

ABSTRACT

Coumarin is a naturally occurring compounds being present in several plants and also can be produced through organic synthetic reactions. In this study, substituted coumarins of 3-acetylcoumarin, 3-acetyl-7-(diethylamino)coumarin, 7-(diethylamino)-3-(1-oxobutyl)coumarin, 3-oxobutyl-3*H*-naphtho[2,1-*b*]pyran-2-one, 6-bromo-3-(1-oxobutyl)coumarin and 8-methoxy-3-(1-oxobutyl)coumarin were synthesized *via* Knoevenagel condensation reaction of respective 2-hydroxy-benzaldehyde derivatives with active methylene group from ethyl acetate or ethyl butyrylacetate under basic condition. Meanwhile, 7-hydroxy-4-methylcoumarin, 4-methyl-2*H*-benzo[*h*]chromen-2-one, 7-hydroxy-4,8-dimethylcoumarin, 7-hydroxy-4-propylcoumarin, 4-propyl-2*H*-benzo[*h*]chromen-2-one, 7-hydroxy-8-methyl-4-propylcoumarin and 7,8-dihydroxy-4-propylcoumarin were prepared through Pechmann condensation reaction by the condensation of respective substituted phenol and β -keto-ester in the presence of sulphuric acid as a catalyst. Further methylation reaction on 7-hydroxy-4-methylcoumarin with iodomethane catalysed by K_2CO_3 gave 7-methoxy-4-methylcoumarin. Modification of hydroxyl group of 7,8-dihydroxy-4-propylcoumarin using butyric anhydride and dry pyridine yielded 7,8-bis-(1-oxobutoxy)-4-propylcoumarin. In addition, 7-hydroxy-4,8-dimethylcoumarin was converted to 7-benzyloxy-4,8-dimethylcoumarin through reaction of benzyl chloride in dry acetone. In addition, 7,8-methylenedioxy-4-propylcoumarin undergoes Williamson etherification reaction which involved S_N2 mechanism between secondary alkyl halide with potassium salt of a phenoxide. All compounds were characterized by spectroscopic techniques using infrared (IR), proton and carbon nuclear magnetic resonance (1H and ^{13}C NMR), and mass spectrometry (MS). The synthesized compounds were tested for their antioxidant and antibacterial activities. It was found that the hydroxylated coumarin derivatives of 7,8-dihydroxy-4-propylcoumarin were tested positive towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with IC_{50} value of 4.09 $\mu g/mL$ while other hydroxylated compounds were inactive. The antibacterial activity show that 6-bromo-3-(1-oxobutyl)coumarin exhibited as strong antibacterial agent against *B. subtilis* bacteria with MIC value 56.25 $\mu g/mL$. 7,8-Methylenedioxy-4-propylcoumarin showed strong activity to all Gram-positive bacteria.

ABSTRAK

Kumarin adalah sebatian semulajadi yang terdapat dalam tumbuhan dan juga boleh dihasilkan melalui tindak balas sintesis organik. Dalam kajian ini, kumarin tertukarganti iaitu 3-asetilkumarin, 3-asetil-7-(dietilamino)kumarin, 7-(dietilamino)-3-(1-oksobutil)kumarin, 3-oksobutil-3*H*-naftol[2,1-*b*]piran-2-on, 6-bromo-3-(1-oksobutil)kumarin dan 8-metoksil-3-(1-oksobutil)kumarin disintesis melalui tindak balas kondensasi Knoevenagel (KCR) menggunakan terbitan 2-hidroksibenzaldehid dengan kumpulan metilena yang aktif daripada etil asetoasetat atau etil butirilasetat dalam keadaan bes. Sementara itu, 7-hidroksi-4-metilkumarin, 4-metilbenzo[*h*]kumarin, 7-hidroksi-4,8-dimetilkumarin, 7-hidroksi-4-propilkumarin, 4-propilbenzo[*h*]kumarin, 7-hidroksi-8-metil-4-propilkumarin dan 7,8-dihidroksi-4-propilkumarin disediakan melalui tindak balas kondensasi Pechmann ke atas fenol tertukarganti dan β -keto ester dengan kehadiran asid sulfurik sebagai mangkin. Tindak balas pemetilan ke atas 7-hidroksi-4-metilkumarin dengan metil iodida dimangkinkan oleh K_2CO_3 memberikan 7-metoksi-4-metilkumarin. Pengubahsuaian ke atas kumpulan hidroksil bagi 7,8-dihidroksi-4-metilkumarin menggunakan butirik anhidrida dan piridina kering menghasilkan 7,8-bis-(1-oksobutoksi)-4-propilkumarin. 7-Hidroksi-4,8-dimetilkumarin telah diubah kepada 7-benziloksi-4,8-dimetilkumarin melalui tindak balas dengan benzil klorida dalam pelarut aseton kering. Di samping itu, 7,8-metilenedioksi-4-propilkumarin disediakan melalui tindak balas eterifikasi Williamson melalui mekanisme S_N2 di antara alkil halida sekunder dan garam kalium fenoksida. Semua hasil tindak balas dicirikan dengan kaedah spektroskopi inframerah (IM), resonans magnet nukleus (RMN 1H and ^{13}C) dan spektrometri jisim (SJ). Sebatian yang disintesis telah melalui ujian aktiviti antioksidan dan antibakteria. Terbitan hidroksi kumarin iaitu 7,8-dihidroksi-4-propilkumarin menunjukkan aktiviti positif terhadap 2,2-difenil-1-pikrilhidrazil (DPPH) dengan nilai IC_{50} 4.09 $\mu g/mL$ manakala sebatian hidroksi yang lain didapati tidak aktif. Hasil ujian aktiviti antibakteria menunjukkan 6-bromo-3-(1-oksobutil)kumarin dikenalpasti sebagai agen antibakteria yang kuat terhadap bakteria *B. subtilis* dengan nilai MIC sebanyak 56.25 $\mu g/mL$. Manakala 7,8-metilenedioksi-4-propilkumarin menunjukkan aktiviti antibakteria yang kuat kepada semua bakteria Gram-positif dalam cerakan antibakteria.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	PREFACE	v
	ABSTRACT	vi
	ABSTRAK	vii
	TABLE OF CONTENTS	viii
	LIST OF TABLES	xii
	LIST OF SCHEMES	xiv
	LIST OF FIGURES	xviii
	LIST OF ABBREVIATIONS	xix
	LIST OF APPENDIX	xxii
1	INTRODUCTION	1
	1.1 Background of Study	1
	1.2 Problem Statement	3
	1.3 Research Objectives	3
	1.4 Scope of Study	4
	1.5 Significant of Study	4

2	LITERATURE REVIEW	5
2.1	Coumarins	5
2.2	Natural Coumarins	6
2.3	Synthetic Coumarins	9
2.3.1	Perkin Condensation Reaction	9
2.3.2	Pechmann Condensation Reaction	10
2.3.3	Knoevenagel Condensation Reaction	16
2.3.4	Kostanecki-Robinson Reaction	20
2.3.5	Claisen Condensation Reaction	22
2.4	Biological Activity of Coumarins	25
3	RESULTS AND DISCUSSION	31
3.1	Introduction	31
3.2	3-Acetylcoumarin (157)	32
3.3	3-Acetyl-7-(diethylamino)coumarin (158)	36
3.4	7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	39
3.5	3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	41
3.6	6-Bromo-3-(1-oxobutyl)coumarin (161)	43
3.7	8-Methoxy-3-(1-oxobutyl)coumarin (162)	45
3.8	7-Hydroxy-4-methylcoumarin (32)	47
3.9	4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	51
3.10	7-Hydroxy-4,8-dimethylcoumarin (164)	53
3.11	7-Hydroxy-4-propylcoumarin (165)	56
3.12	4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	58
3.13	7-Hydroxy-8-methyl-4-propylcoumarin (167)	60
3.14	7,8-Dihydroxy-4-propylcoumarin (168)	62
3.15	Derivatization of Synthesized Coumarins	64
3.15.1	7-Methoxy-4-methylcoumarin (169)	65

3.15.2	7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	67
3.15.3	7-Benzoyloxy-4,8-dimethylcoumarin (171)	70
3.15.4	7,8-Methylenedioxy-4-propylcoumarin (172)	73
3.16	Antioxidant Activity	76
3.16.1	Free Radical Scavenging Activity (DPPH)	77
3.17	Antibacterial Activity	78
4	RESEARCH METHODOLOGY	82
4.1	General Instruments and Apparatus	82
4.2	Chemicals	83
4.3	Experimental	83
4.3.1	3-Acetylcoumarin (157)	84
4.3.2	3-Acetyl-7-(diethylamino)coumarin (158)	85
4.3.3	7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	85
4.3.4	3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	86
4.3.5	6-Bromo-3-(1-oxobutyl)coumarin (161)	87
4.3.6	8-Methoxy-3-(1-oxobutyl)coumarin (162)	87
4.4	Synthesis of Coumarin and Its derivatives through Pechmann Condensation Reaction	88
4.4.1	7-Hydroxy-4-methylcoumarin (32)	89
4.4.2	4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	89
4.4.3	7-Hydroxy-4,8-dimethylcoumarin (164)	90
4.4.4	7-Hydroxy-4-propylcoumarin (165)	91
4.4.5	4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	91
4.4.6	7-Hydroxy-8-methyl-4-propylcoumarin (167)	92
4.4.7	7,8-Dihydroxy-4-propylcoumarin (168)	93
4.5	Derivatization Reaction	93

4.5.1	7-Methoxy-4-methylcoumarin (169)	93
4.5.2	7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	94
4.5.3	7-Benzyloxy-4,8-dimethylcoumarin (171)	95
4.5.4	7,8-Methylenedioxy-4-propylcoumarin (172)	96
4.6	Antioxidant Assay	96
4.7	Antibacterial Assay	98
4.7.1	Equipments, Chemicals and Bacteria	98
4.7.2	Microorganism and Culture Media	98
4.7.3	Disc Diffusion Methods (DDM)	99
4.7.4	Minimum Inhibition Concentration (MIC)	100
4.7.5	Minimum Bactericidal Concentration (MBC)	101
5	CONCLUSION AND RECOMMENDATIONS	102
5.1	Conclusion	102
5.2	Recommendations	104
	REFERENCES	105
	APPENDICES	117
	PUBLICATION	195

LIST OF TABLES

TABLE NO.	TITLE	PAGE
1.1	The main structural features and example of four main coumarin subtypes	2
3.1	^1H and ^{13}C NMR Data for Acetylcoumarin (157)	36
3.2	^1H and ^{13}C NMR Data for 3-Acetyl-7-(diethylamino)-coumarin (158)	38
3.3	^1H and ^{13}C NMR Data for 7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	40
3.4	^1H and ^{13}C NMR Data for 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	43
3.5	^1H and ^{13}C NMR Data for 6-Bromo-3-(1-oxobutyl)-coumarin (161)	44
3.6	^1H and ^{13}C NMR Data for 8-Methoxy-3-(1-oxobutyl)-coumarin (162)	46
3.7	^1H and ^{13}C NMR Data for 7-Hydroxy-4-methylcoumarin (32)	49
3.8	^1H and ^{13}C NMR Data for 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	53
3.9	^1H and ^{13}C NMR Data for 7-Hydroxy-4,8-dimethyl-coumarin (164)	55
3.10	^1H and ^{13}C NMR Data for 7-Hydroxy-4-propylcoumarin (165)	58

3.11	¹ H and ¹³ C NMR Data for 4-propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	60
3.12	¹ H and ¹³ C NMR Data for 7-Hydroxy-8-methyl-4-propyl-coumarin (167)	62
3.13	¹ H and ¹³ C NMR Data for 7,8-Dihydroxy-4-propyl-coumarin (168)	64
3.14	¹ H and ¹³ C NMR Data for 7-Methoxy-4-methylcoumarin (169)	67
3.15	¹ H and ¹³ C NMR Data for 7,8-Bis-(1-oxobutoxy)-4-propyl-coumarin (170)	70
3.16	¹ H and ¹³ C NMR Data for 7-Benzoyloxy-4,8-dimethyl-coumarin (171)	73
3.17	¹ H and ¹³ C NMR Data for 7,8-Methylenedioxy-4-propyl-coumarin (172)	75
3.18	Antioxidant Assay of Coumarin Derivatives	78
3.19	Minimum Inhibitory Concentration (MIC) of Tested Compounds	80
3.20	Minimum Bactericidal Concentration (MBC) of Tested Compounds	81
4.1	Percentage of Inhibition of All Samples	96

LIST OF SCHEMES

TABLE NO.	TITLE	PAGE
2.1	Synthesis of coumarin by Perkin condensation reaction	10
2.2	Synthesis of 3-Cyanocoumarin (30) in the presence of propylphosphonic anhydride (T3P)	10
2.3	Synthesis of 7-Hydroxy-4-methylcoumarin (32) undergoes non- environmental friendly condition	11
2.4	Synthesis of 7-Hydroxy-4-methylcoumain (32) undergoes solvent free heterogeneous catalytic conditions	11
2.5	Synthesis of 4-Methylcoumarin and its derivatives in the presence of [TMPSA][HSO ₄] ionic liquid	12
2.6	Synthesis of 7,8-Dihydroxy-4-methylcoumarin (45) and its derivatives of acetylation and butylation	13
2.7	Synthesis of 4-Methylcoumarin derivatives (49-51) under Pechmann condensation reaction	14
2.8	Synthesis of 4-Methylcoumarin derivatives (52-56) by acetylation and propylation	14
2.9	Synthesis of 4-Methylcoumarin (57) in presence of PVPP-BF ₃ as a catalyst	15
2.10	Synthesis of 7-Hydroxy-4-methylcoumarin (32) in the presence of InCl ₃ added as catalyst	16
2.11	Liquid phase Pechmann condensation of resorcinol and ethyl acetoacetate	16

2.12	Knoevenagel condensation reaction assisted by microwave heating in the presence of $ZrOCl_3$ as a catalyst under solvent free conditions	17
2.13	Synthesis of coumarin 3-carboxylic acid (72)	18
2.14	Synthesis of coumarin 3-carboxylic acid derivatives (76-85) under Knoevenagel condensation reaction	18
2.15	Synthesis of coumarin-3-carboxylic acid and their ester derivatives by condensation of salicylaldehyde with dialkyl malonate or Meldrum's acid	19
2.16	Synthesis of coumarin-3-carboxylic acid and their ester derivatives by condensation of 2-hydroxy-1-naphthaldehyde with dialkyl malonate or Meldrum's acid	20
2.17	Synthesis of 3 and 4-substituted coumarins (97-99) by Kostanecki-Robinson Reaction	21
2.18	The Kostanecki-Robinson Acylation of 5-Hydroxy-6-acetyl-4-methyl- coumarin (102)	22
2.19	Synthesis of coumarin 4-Hydroxycoumarin (104) by Claisen Condensation Reaction	22
2.20	Synthesis of dimers of 4-hydroxycoumarin (106-114)	23
2.21	Synthesis of tetramers of 4-hydroxycoumarin (116-117)	23
2.22	Synthesis of 4-Hydroxycoumarin Derivatives (122-125)	24
2.23	Synthesis of 4-hydroxycoumarin derivatives (126, 128)	25
2.24	Synthesis of coumarin derivatives (141-154) by alkylation, acetylation and nitration reaction.	33
3.1	Synthesis of 3-Acetylcoumarin (157)	33
3.2	Enolate formation using a secondary amine	34
3.3	The mechanism for the synthesis of compound (157)	36
3.4	Synthesis of 3-Acetyl-7-(diethylamino)coumarin (158)	39

3.5	Attempted 3-Acetyl-7-(diethylamino)coumarin (158) to 3-Cinnamoyl-7-(diethylamino)coumarin (coumarin- chalcone) (175)	39
3.6	Synthesis of 7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	39
3.7	Attempted Reduction of 7-(Diethylamino)-3-(1-oxobutyl)-coumarin (159) to 7-(Diethylamino)-3-(1-hydroxybutyl)-coumarin (178)	41
3.8	Synthesis of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1-b]pyran-2-one (160)	42
3.9	Fragmentation Pattern of Compound (160)	42
3.10	Synthesis of 6-Bromo-3-(1-oxobutyl)coumarin (161)	44
3.11	Synthesis of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	45
3.12	Attempted Nitration of 8-Methoxy-3-(1-oxobutyl)coumarin (162) to furnish 8-Methoxy-5-nitro-3-(1-oxobutyl)-coumarin (182)	47
3.13	Synthesis of 7-Hydroxy-4-methylcoumarin (32)	47
3.14	The mechanism for the synthesis of compound (32)	48
3.15	An attempted to convert 7-Hydroxy-4-methylcoumarin (32) to 7-hydroxy-4-methyl-3-(1,1-dimethyl-3-oxobutyl)-coumarin (185)	50
3.16	An attempted to convert 7-hydroxy-4-methylcoumarin (32) to 3,3'-[(benzyl)methylene]bis(7-hydroxy-4-methyl-coumarin) (186)	51
3.17	Synthesis of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	51
3.18	Synthesis of 7-Hydroxy-4,8-dimethylcoumarin (164)	53
3.19	An attempted to convert 7-hydroxy-4,8-dimethylcoumarin (164) to 4,4-dimethyl-2 <i>H</i> ,8 <i>H</i> -benzo[1,2- <i>b</i> :4,5- <i>b'</i>]dipyran-2,8-dione (189)	55

3.20	An attempted on the bromination of 7-hydroxy-4-dimethyl-coumarin (164) to 6-bromo-7-butoxy-4,8-dimethyl-coumarin (190)	56
3.21	Synthesis of 7-Hydroxy-4-propylcoumarin (165)	56
3.22	Synthesis of 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	58
3.23	Synthesis of 7-Hydroxy-8-methyl-4-propylcoumarin (167)	61
3.24	Synthesis of 7,8-Dihydroxy-4-propylcoumarin (168)	63
3.25	Synthesis of 7-Methoxy-4-methylcoumarin (169)	65
3.26	The mechanism for the synthesis of 7-Methoxy-4-methyl-coumarin (169)	66
3.27	Synthesis of 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	68
3.28	Formation of butyryl acetate ion (193) and pyridinium butyrylacetate ion (194)	68
3.29	The mechanism of esterification reaction to form compound (170)	69
3.30	Synthesis of 7-Benzoyloxy-4,8-dimethylcoumarin (171)	71
3.31	The mechanism of benzylation reaction for the formation of compound (171)	71
3.32	Synthesis of 7,8-Methylenedioxy-4-propylcoumarin (172)	74
3.33	The mechanism of ketalation reaction for the formation of compound (172)	74
3.34	The Reduction of DPPH	77

LIST OF FIGURES

FIGURES	TITLE	PAGE
4.1	The arrangement of the samples and controls disc in disc diffusion method	100

LIST OF ABBREVIATIONS

AA	arachidonic acid
AIDS	acquired immune deficiency syndrome
ATR	attenuated total reflectance
BaCl ₂	barium (II) chloride
¹³ C	carbon-13
CaCO ₃	calcium carbonate
CaCl ₂	calcium chloride
CDCl ₃	deuterated chloroform
CD ₃ COCD ₃	deuterated acetone
CD ₃ OD	deuterated methanol
CH ₂ I ₂	diiodomethane
CH ₃ I	iodomethane
CIMS	Chemical Impact Mass Spectrometry
d	doublet
dd	doublet of doublet
DDM	disc diffusion method
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EIMS	Electron Impact Mass Spectrometry
FRAP	Ferric Reducing/ Antioxidant Power

hr	hour
^1H	proton
HIV	human immunodeficiency virus
HNO_3	nitric acid
H_2SO_4	sulphuric acid
Hz	hertz
I%	percentage of inhibition
IC_{50}	inhibition concentration at 50%
IR	infrared
InCl_3	indium (III) chloride
<i>J</i>	coupling constant
KBr	potassium bromide
KCR	Knoevenagel condensation reaction
K_2CO_3	potassium carbonate
lit	literature
<i>m/z</i>	mass to charge ratio
m	multiplet
m.p	melting point
m-ZrP	mesoporous zirconium phosphate
MBC	minimum bactericidal concentration
MIC	minimum inhibition concentration
MS	mass spectrometry
n-BuOAc	n-butyl acetate
NA	nutrient agar
NB	nutrient broth
NMR	nuclear magnetic resonance
ORAC	Oxygen Radical Absorbance Capacity
PAF	platelet activating factor
PSC	Peroxyl Radical Scavenging Capacity

PVPP-BF ₃	polyvinylpyrrolidone-bound boron trifluoride
q	quartet
s	singlet
S _N 2	Second-Order Nucleophilic Substitution reaction
SD	standard deviation
SS	streptomycin sulphate
r.t	room temperature
R _f	retention factor
t	triplet
T3P	propylphosphonic anhydride
TBATB	<i>n</i> -tetrabutylammonium tribromide
TEAC	Trolox Equivalent Antioxidant Capacity
TGMT	1,1,3,3,- <i>N,N,N',N'</i> -tetramethylguanidinium trifluoroacetate ionic liquid
THF	tetrahydrofuran
TLC	thin layer chromatography
[TMPSA][HSO ₄]	<i>N,N,N</i> -Trimethyl- <i>N</i> -propanesulfonic acid ammonium hydrogen sulfate
TOSC	Total Oxyradical Scavenging Capacity
TRAP	Total Radical-Trapping Antioxidant Parameter
TSIL	task-specific ionic liquids
UV-Vis	ultraviolet visible
ZrOCl ₃	zirconium salt
Zr-TMS	zirconia based transition metal oxide mesoporous molecular sieve
α	alpha
β	beta
δ	chemical shift

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
1	IR Spectrum of 3-Acetylcoumarin (157)	118
2	¹ H NMR Spectrum of 3-Acetylcoumarin (157)	119
3	¹³ C NMR Spectrum of 3-Acetylcoumarin (157)	120
4	CIMS spectrum of 3-Acetylcoumarin (157)	121
5	IR Spectrum of 3-Acetyl-7-(diethylamino)coumarin (158)	122
6	¹ H NMR Spectrum of 3-Acetyl-7-(diethylamino)coumarin (158)	123
7	Expanded ¹ H NMR Spectrum of 3-Acetyl-7-(diethylamino)coumarin (158)	124
8	¹³ C NMR Spectrum of 3-Acetyl-7-(diethylamino)coumarin (158)	125
9	CIMS Spectrum of 3-Acetyl-7-(diethylamino)coumarin (158)	126
10	IR Spectrum of 7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	127
11	¹ H NMR Spectrum of 7-(Diethylamino)-3-(1-oxobutyl)-coumarin (159)	128
12	Expanded ¹ H NMR Spectrum of 7-(Diethylamino)-3-(1-oxobutyl)coumarin (159)	129
13	¹³ C NMR Spectrum of 7-(Diethylamino)-3-(1-oxobutyl)-coumarin (159)	130

14	CIMS Spectrum of 7-(Diethylamino)-3-(1-oxobutyl)-coumarin (159)	131
15	IR Spectrum of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	132
16	¹ H NMR Spectrum of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	133
17	Expanded ¹ H NMR Spectrum of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	134
18	¹³ C NMR Spectrum of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	135
19	EIMS spectrum of 3-Oxobutyl-3 <i>H</i> -naphtho[2,1- <i>b</i>]pyran-2-one (160)	136
20	IR Spectrum of 6-Bromo-3-(1-oxobutyl)coumarin (161)	137
21	¹ H NMR Spectrum of 6-Bromo-3-(1-oxobutyl)coumarin (161)	138
22	Expanded ¹ H NMR spectrum of 6-Bromo-3-(1-oxobutyl)-coumarin (161)	139
23	¹³ C NMR Spectrum of 6-Bromo-3-(1-oxobutyl)coumarin (161)	140
24	EIMS Spectrum of 6-Bromo-3-(1-oxobutyl)coumarin (161)	141
25	IR Spectrum of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	142
26	¹ H NMR Spectrum of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	143
27	¹³ C NMR Spectrum of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	144
28	Expanded ¹³ C NMR Spectrum of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	145
29	CIMS Spectrum of 8-Methoxy-3-(1-oxobutyl)coumarin (162)	146

30	IR Spectrum of 7-Hydroxy-4-methylcoumarin (32)	147
31	¹ H NMR Spectrum of 7-Hydroxy-4-methylcoumarin (32)	148
32	¹³ C NMR Spectrum of 7-Hydroxy-4-methylcoumarin (32)	149
33	EIMS Spectrum of 7-Hydroxy-4-methylcoumarin (32)	150
34	IR Spectrum of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	151
35	¹ H NMR Spectrum of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	152
36	Expanded ¹ H NMR Spectrum of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	153
37	¹³ C NMR Spectrum of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	154
38	EIMS Spectrum of 4-Methyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (163)	155
39	IR Spectrum for 7-Hydroxy-4,8-dimethylcoumarin (164)	156
40	¹ H NMR Spectrum for 7-Hydroxy-4,8-dimethylcoumarin (164)	157
41	¹³ C NMR Spectrum for 7-Hydroxy-4,8-dimethylcoumarin (164)	158
42	EIMS Spectrum for 7-Hydroxy-4,8-dimethylcoumarin (164)	159
43	IR Spectrum for 7-Hydroxy-4-propylcoumarin (165)	160
44	¹ H NMR Spectrum for 7-Hydroxy-4-propylcoumarin (165)	161
45	Expanded ¹ H NMR Spectrum for 7-Hydroxy-4-propylcoumarin (165)	162
46	¹³ C NMR Spectrum for 7-Hydroxy-4-propylcoumarin (165)	163

47	CIMS Spectrum for 7-Hydroxy-4-propylcoumarin (165)	164
48	IR Spectrum for 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	165
49	¹ H NMR Spectrum for 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	166
50	Expanded ¹ H NMR Spectrum for 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	167
51	¹³ C NMR Spectrum for 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	168
52	EIMS Spectrum for 4-Propyl-2 <i>H</i> -benzo[<i>h</i>]chromen-2-one (166)	169
53	IR Spectrum for 7-Hydroxy-8-methyl-4-propylcoumarin (167)	170
54	¹ H NMR Spectrum for 7-Hydroxy-8-methyl-4-propylcoumarin (167)	171
55	¹³ C NMR Spectrum for 7-Hydroxy-8-methyl-4-propylcoumarin (167)	172
56	CIMS Spectrum for 7-Hydroxy-8-methyl-4-propylcoumarin (167)	173
57	IR Spectrum for 7,8-Dihydroxy-4-propylcoumarin (168)	174
58	¹ H NMR Spectrum for 7,8-Dihydroxy-4-propylcoumarin (168)	175
59	¹³ C NMR Spectrum for 7,8-Dihydroxy-4-propylcoumarin (168)	176
60	EIMS Spectrum for 7,8-Dihydroxy-4-propylcoumarin (168)	177
61	IR Spectrum for 7-Methoxy-4-methylcoumarin (169)	178
62	¹ H NMR Spectrum for 7-Methoxy-4-methylcoumarin (169)	179

63	¹³ C NMR Spectrum for 7-Methoxy-4-methylcoumarin (169)	180
64	EIMS Spectrum for 7-Methoxy-4-methylcoumarin (169)	181
65	IR Spectrum for 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	182
66	¹ H NMR Spectrum for 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	183
67	Expanded ¹ H NMR Spectrum for 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	184
68	¹³ C NMR Spectrum for 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	185
69	¹³ C and DEPT NMR Spectra for 7,8-Bis-(1-oxobutoxy)-4-propylcoumarin (170)	186
70	IR Spectrum for 7-Benzyloxy-4,8-dimethylcoumarin (171)	187
71	¹ H NMR Spectrum for 7-Benzyloxy-4,8-dimethylcoumarin (171)	188
72	Expanded ¹ H NMR Spectrum for 7-Benzyloxy-4,8-dimethylcoumarin (171)	189
73	¹³ C NMR Spectrum for 7-Benzyloxy-4,8-dimethylcoumarin (171)	190
74	IR Spectrum of 7,8-Methylenedioxy-4-propylcoumarin (172)	191
75	¹ H NMR Spectrum for 7,8-Methylenedioxy-4-propylcoumarin (172)	192
76	Expanded ¹ H NMR Spectrum for 7,8-Methylenedioxy-4-propylcoumarin (172)	193
77	¹³ C NMR Spectrum for 7,8-Methylenedioxy-4-propylcoumarin (172)	194

CHAPTER 1

INTRODUCTION

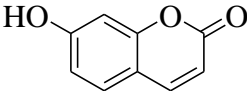
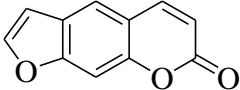
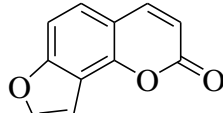
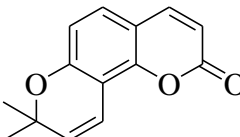
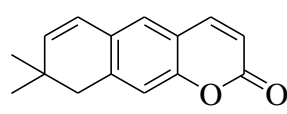
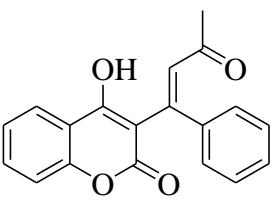
1.1 Introduction

Coumarins (benzopyrones) are simple molecules that contain two structures of six member heterocyclic rings with two oxygen atoms. The coumarins can be classified as simple coumarin, furanocoumarins, pyranocoumarins and coumarins substituted in the pyrone ring (**Table 1.1**) [1]. Simple coumarins can undergoes hydroxylation, alkoxylation and alkylation to form its derivatives. For furanocoumarins, these compounds consist of five-member furan ring attached to the coumarin structure. Pyranocoumarins is a compound that contained a linear or angular type with substituents on benzene and pyrone rings.

Coumarins are naturally occurring important plant constituents and occupy unique in natural and organic synthesis chemistry [2]. Coumarin can be found in several plants especially in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), woodruff (*Galium odoratum*), mullein (*Verbascum* spp.), and sweet grass (*Hierochloe odorata*). Coumarin has a sweet scent and had been recognised as the scent of newly-mown hay. Because of the sweet scent, coumarin has been used in perfumes since 1882 [3]. Coumarins also can be produced through organic synthetic reactions although it can be found naturally in several green plants. Coumarins and its derivatives can be synthesized through Knoevenagel condensation reaction (KCR), Pechmann condensation reaction, Perkin

reaction, Wittig reaction, Claisen, Reformatsky and Kontanecki-Robinson reaction [4].

Table 1.1: The main structural features and examples of four subtype of coumarin.

Classification	Features	Examples
Simple coumarins	Hydroxylated, alkoxyated or alkylated on benzene ring	 7-Hydroxycoumarin
Furanocoumarins	5-membered furan ring attached to benzene ring Linear or angular	  Psoralen Angelicin
Pyranocoumarins	6-membered pyran ring attached to benzene ring Linear or angular	  Seselin Xanthyletin
Pyrone-substituted coumarins	Substitution on pyrone ring, often at 3-C or 4-C positions	 Warfarin

Nowadays, coumarins play an important role as food constituents [5], and dye-sensitized solar cells [6]. Coumarins also used as additives to cigarettes [7]. In addition, coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances [8]. Coumarin and its derivatives were successfully made great interesting targets for organic chemist due to their chemical properties. The pharmacological and biochemical properties and also the applications of therapeutics will be affected due

to the different position of coumarin substitution. Thus, it is utmost important to synthesis coumarins and their derivatives through a simple and effective method.

1.2 Problem Statements

Coumarins can be isolated from plants naturally but with the low percentage of yields. Thus, this problem limits the use of coumarins in various applications especially for bioactivity purposes. Many synthesis methods have been developed to synthesize the coumarins and its derivatives such as Knoevenagel and Pechmann condensation reactions. Both of these methods were used in this study which offered convenience synthetic procedures to access coumarin derivatives which are not found naturally. The synthetic compounds may provide interesting biological properties especially in antioxidant and antibacterial studies.

1.3 Research Objectives

The objectives of this study are:

1. To synthesize and characterize coumarin derivatives
2. To carry out derivatization reaction of the synthesized coumarin.
3. To screen the antioxidant and antibacterial activities of coumarin derivatives.

1.4 Scope of Study

In this study, two methods were used to synthesize coumarin derivatives which are Knoevenagel condensation reaction (KCR) and Pechmann condensation reaction. Coumarin derivatives were synthesized by KCR which involved the condensation of an aldehyde with a compound containing an active methylene group in the presence of catalytic amount of base. Coumarin derivatives also can be synthesized through Pechmann condensation reaction which involved the condensation of substituted phenols with β -ketoester in the presence of an acid catalyst. The synthesized coumarins were undergoes derivatization reaction to synthesize several type of coumarin derivatives such as methylation, esterification, benzylation and ketalation. All compounds were characterized by spectroscopic techniques using infrared (IR), proton and carbon-13 nuclear magnetic resonance (^1H and ^{13}C NMR), and mass spectrometry (MS). Screening of antioxidant and antibacterial activities for the synthesized coumarin derivatives were carried out.

1.5 Significant of Study

Coumarins have strong biochemical and pharmacological properties. Coumarins and its derivatives are very useful in medicine field such as for the treatment of lymphoedema, and in treatment of cancer (breast, lung and kidney cancer) [9]. This research is vital to synthesize the coumarin compounds by using KCR and Pechmann reaction. These will provide the alternative way to afford coumarin and its derivatives as compared to natural resources. Screening of biological activities such as antioxidant and antibacterial activities for the synthesized coumarin derivatives were carried out indicated the useful of these compounds.

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