SYNTHESIS, CHARACTERIZATION AND MICROBIAL ACTIVITY OF NEW THIAZOLE, THIAZOLIDINONE, THIADIAZOLE AND PHENYLACRYLOHYDRAZIDE SUBSTITUTED COUMARIN DERIVATIVES

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UNIVERSITI SAINS MALAYSIA

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By

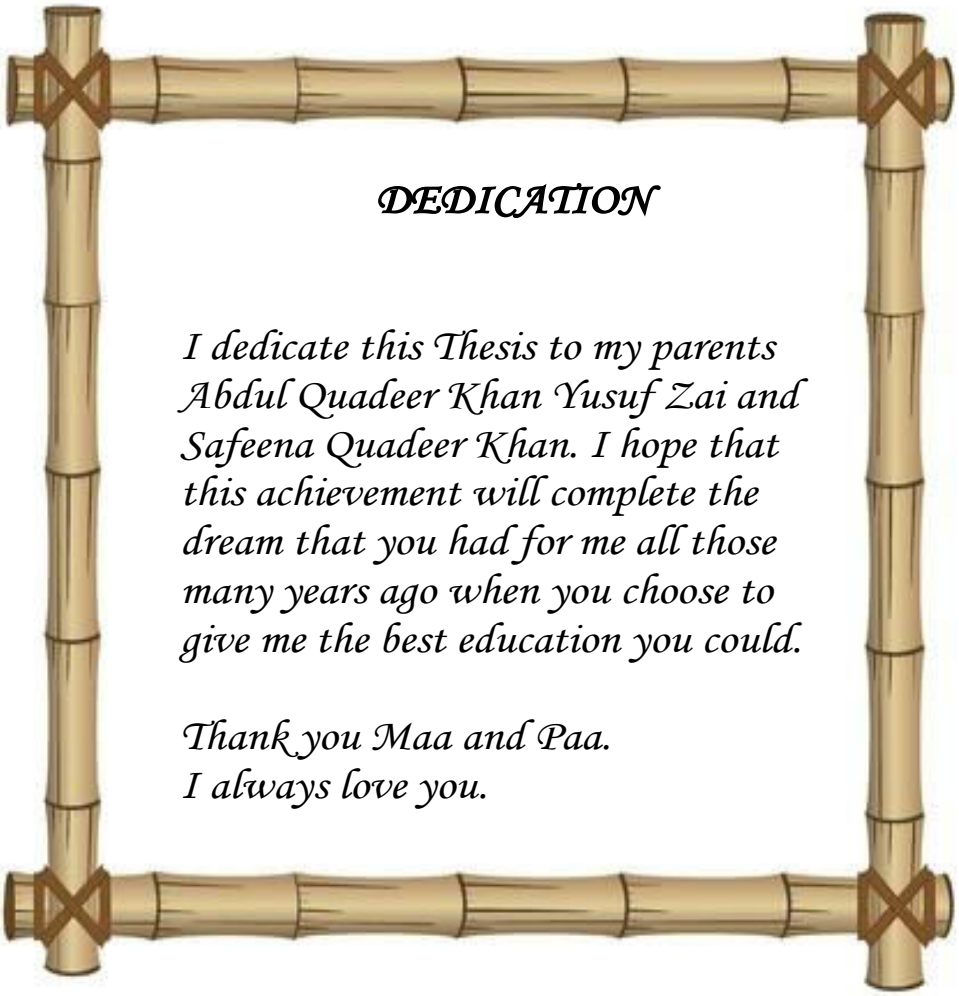
SAMINA KHAN YUSUF ZAI

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

JULY 2015

Dear Husband Shaheen, I owe you a debt of gratitude for all that you have done for me. I thank the Almighty for bringing You in my Life.

Thank you for being there Always.



DEDICATION

I dedicate this Thesis to my parents Abdul Quadeer Khan Yusuf Zai and Safeena Quadeer Khan. I hope that this achievement will complete the dream that you had for me all those many years ago when you choose to give me the best education you could.

Thank you Maa and Paa.
I always love you.

ACKNOWLEDGEMENT

First and above all, I praise God the almighty for providing me this opportunity and granting me the strength and capability to proceed successfully during my studies. Indeed, without His Help and Will nothing is accomplished. Life blessed me with the opportunity to meet my wonderful supervisor Professor Hasnah Osman. This thesis is a small tribute to an exceptional lady from a student, still anxious to learn from her. I owe my deepest gratitude to my co-supervisor Professor Othman Sulaiman from School of Industrial Technology, USM for his valuable suggestions, motivation and all his help. I would also like to thanks my field co-supervisor Dr. Jualang Azlan Gansau from School of Science and Natural Resources, University Malaysia Sabah for providing me all the necessary lab facilities to conduct my antibacterial assay during the stay of my studies in UMS. I wish to thank Dr. Suriyati Mohammad from School of Biological Sciences, USM for providing me laboratory facilities and useful suggestions to conduct my anti-tuberculosis assay. I wish to acknowledge Mr. Zahari Othman for his knowledge and technical support in NMR. I would like to thanks Dr. Abdul Razak Ibrahim and Mr. Mustaqim Rosli from School of Physics, USM for their support in X-ray crystallography data acquisition. I am heartily thankful to government of Malaysia, Institute of Postgraduate Studies, University Sains Malaysia and School of Chemical Sciences for giving me this golden chance of study and providing me all the necessary facilities for my research work. I am grateful to the many Malaysians who over these years have welcomed me into their homes and offices as friend. The understanding and friendship gained by them formed the vital background to my study. The warm support of all my friends in Malaysia enabled me to complete this thesis and have a wonderful time along the way. I wish I could mention each individually. I would also like to acknowledge Mr.

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Samina Khan Yusuf Zai

July 2015

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

Chemicals

ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonicacid)
ADC	Albumin, dextrose and catalase
$BaCl_2$	Barium chloride
$CH_3COOCOCH_3$	Acetic anhydride
$ClCH_2COOH$	Chloroacetic acid
CO_2	Carbon dioxide
DPPH	1,1-Diphenylpicrylhydrazyl radical
FRAP	Ferric reducing antioxidant power
FTC	Ferric thiocyanate
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
KBr	Potassium bromide
LDH	Lactate dehydrogenase
MABA	Micro plate alamar blue assay
MHB	Muller Hinton broth
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide
NA	Nutrient agar
NB	Nutrient broth
NaOAc	Sodium acetate
$NH_2NHCOCH_2CN$	Cyanoacetohydrazide
NH_4OH	Ammonium hydroxide
OADC	Oleic, albumin, dextrose and catalase

SDH	Succinate dehydrogenase
TTC	2,3,5-triphenyltetrazolium chloride
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulpho phenyl)-2*H*-tetrazolium-5-carboxanilide

Instrument and Techniques

CHN	Carbon, hydrogen and nitrogen
COSY	Correlated spectroscopy
^{13}C NMR	Carbon nuclear magnetic resonance
HMBC	Heteronuclear multiple bond connectivity
HMQC	Heteronuclear multiple quantum coherence
HPLC	High performance liquid chromatography
^{1}H NMR	Proton nuclear magnetic resonance
IR	Infra red
LC-MS	Liquid chromatography mass spectrometry
ORTEP	Oak ridge thermal ellipsoid plot
SAR	Structure-activity relationship
TEMA	Tetrazolium micro plate assay
TLC	Thin layer chromatography

Microbes

A. aerogenes	*Enterobacter aerogenes*
E. coli	*Escherichia coli*
M. tuberculosis	*Mycobacterium tuberculosis*
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
S. typhi	*Salmonella typhi*

Solvents

$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
C_6H_6	Benzene
DMSO	Dimethyl sulphoxide
DMSO-d_6	Deuterated dimethyl sulphoxide
EtOAc	Ethyl acetate
EtOH	Ethanol
H_2O	Water

Symbols

α	alpha
amu	atomic mass unit
β	beta
γ	gamma
°C	degree celsius
CFU	colony forming unit
cm^{-1}	per centimeter
δ	chemical shift in delta
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
1D	one dimensional
2D	two dimensional
g	gram

g/L	gram/liter
HSAB	hard and soft acid and base
Hz	hertz
IC_{50}	concentration required inhibiting 50% of effect
J	coupling constant
L	liter
λ_{max}	lambda (maximum wavelength)
m	multiplet
MHz	mega hertz
MIC	minimum inhibitory concentration
min	minutes
mL	milliliter
mol	mole
MP	melting point
mp	melting point
m/z	mass/charge
µg	microgram
µM	micro molar
µg/mL	micro gram per milliliter
nm	nanometer
ppm	parts per million
%	percentage
s	singlet
t	triplet
TB	tuberculosis

td	triplet of doublet
TMS	tetramethylsilane
v/v	volume/volume
w/v	weight/volume
w/w	weight/weight

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2- Yusufzai, S. K., Osman, H., Rahim, A. S. A., Arshad, S., Razak, I. A. (2012). 3*β*-Chloro-5*α*-cholestan-6-one. *Acta Cryst., E68*, o1211-o1212.

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4- Yusufzai, S. K., Osman, H., Sulaiman, O., Gansau, J. A., Razak, I. A. (2013). Synthesis and characterization of novel thiazole coumarin derivatives. The 11th Annual seminar on science and technology (S & T), University Malaysia Sabah, 30 Nov-1st Dec, 2013 at University Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

SINTESIS, PENCIRIAN DAN AKTIVITI MIKROBIAL THIAZOL, THIAZOLIDINON, THIADIAZOL DAN FENILAKRILOHIDRAZID TERCANTUMKAN TERBITAN KUMARIN BARU

ABSTRAK

Thiazol, thiazolidinon, thiadiazol, fenilakrilohidrazid dan kumarin merupakan sebahagian daripada kelas sebatian heterosiklik yang mempunyai kepentingan dalam bidang perubatan dan farmakologi. Dalam kajian ini enam siri daripada 67 sebatian kumarin baru yang aktif secara biologi telah disintesis dengan mencantumkan unit thiazol, thiazolidinon, thiadiazol dan fenilakrilohidrazid ke dalam rangka kumarin untuk meningkatkan kegunaannya sebagai agen terapeutik yang penting. Semua terbitan yang telah disintesis dicirikan menggunakan teknik spektroskopik seperti IR, 1D NMR (^{1}H dan ^{13}C) dan analisis CHN bersama LC-MS. Struktur terbitan terpilih telah dipastikan lebih dengan lanjut menggunakan teknik spektroskopik 2D NMR (COSY, HMQC dan HMBC). Di samping itu, analisis pembelauan X-Ray telah digunakan bagi mengesahkan struktur sebatian yang diperolehi adalah kristal tunggal. Struktur semua bahan permulaan yang disintesis telah disahkan dengan membandingkan data spektroskopi tersebut dengan yang telah dilaporkan dalam literatur. Sukatan takat lebur telah dijalankan kepada semua sebatian bagi memeriksa ketulenan mereka. Semua terbitan yang disintesis telah melalui saringan *in vitro* bagi aktiviti antibakteria terhadap dua bakteria jenis Gram-positif *Streptococcus pneumoniae* dan *Staphylococcus aureus* dan tiga bakteria jenis Gram-negatif *Escherichia coli*, *Enterobacter aerogenes* dan *Salmonella typhi*, termasuklah *Mycobacterium tuberculosis* bakteria penyebab penyakit tuberkulosis. Aktiviti signifikan terhadap *Mycobacterium tuberculosis* telah ditunjukkan oleh hampir kesemua sebatian. Perencatan terkuat telah ditunjukkan oleh **29g** sebatian terhalogen

dengan nilai MIC terendah 60.0 μM, manakala analognya **29f** dan kumarin lain **27b**, **30a** dan **26** juga mendedahkan aktiviti yang baik, masing-masing dengan nilai-nilai MIC 83, 93, 83 dan 93 μM berbanding dengan ubat kawalan, Isoniazid. Selain itu, dalam aktiviti perencatan anti-bakteria yang paling kuat terhadap strain bakteria yang disebutkan di atas diperhatikan lagi menggunakan kumpulan sebatian terhalogen (**27b**, **28b**, **28i**, **28p**, **29d**, **29h**, **30b**, **31b** dan **32d**), dengan nilai-nilai MIC antara 34 hingga 233 μM berbanding dengan ubat standard streptomycin, kanamycin dan vancomycin. Terbitan kumarin lain juga didapati mempamerkan aktiviti-aktiviti anti bakteria yang ketara. Keputusan kajian ini menyimpulkan potensi penggunaan terbitan kumarin, dalam bidang perubatan dan ubatan.

SYNTHESIS, CHARACTERIZATION AND MICROBIAL ACTIVITY OF NEW THIAZOLE, THIAZOLIDINONE, THIADIAZOLE AND PHENYL ACRYLOHYDRAZIDE SUBSTITUTED COUMARIN DERIVATIVES

ABSTRACT

Thiazoles, thiazolidinones, thiadiazoles, phenylacrylohydrazides and coumarins, are few of the very important classes of heterocyclic compounds having great medicinal and pharmacological importance. In the present study, six series of 67 new biologically active coumarin derived compounds have been synthesized by substituting thiazole, thiazolidinone, thiadiazole and phenylacrylohydrazide units into the coumarin core, in order to increase their use as important therapeutic agents. The structures of all the new compounds were characterized by spectroscopic techniques such as, IR, 1D NMR (^{1}H and ^{13}C) and CHN analysis together with LC-MS. The structures of the selective compounds from each series were further confirmed by 2D NMR (COSY, HMQC and HMBC) spectroscopic techniques. In addition to this X-Ray diffraction analysis was used to confirm, the structures for those compounds obtained as single crystals. The structures of all the synthesized starting materials were confirmed by the comparison of their spectroscopic data with those already reported in literature. Melting point measurements was done for all the synthesized compounds, in order to check their purity. All the synthesized coumarin analogues were screened *in vitro* for their antibacterial activity against two Gram-positive bacteria *Streptococcus pneumoniae* and *Staphylococcus aureus* and three Gram-negative bacteria *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella typhi*, including *Mycobacterium tuberculosis*, a bacterium which causes tuberculosis disease. Significant activity against *Mycobacterium tuberculosis* was shown by almost all the compounds. Strongest inhibition was shown by the halogenated

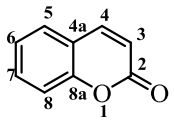
compound **29g** with the lowest MIC value of 60.0 μM, while its analogue **29f** and the other coumarins **27b, 30a** and **26** also revealed good activities with MIC values of 83, 93, 83 and 93 μM, respectively as compared to that of the control drug, Isoniazid. Moreover, in antibacterial activity the strongest inhibition against the aforementioned bacteria strains were observed again by the group of halogenated compounds (**27b**, **28b**, **28i**, **28p**, **29d, 29h, 30b, 31b,** and **32d**), with MIC values ranging from 34 to 233 μM as compared to those of the standard drugs streptomycin, kanamycin and vancomycin. Rest other coumarin derivatives were also found to exhibit significant anti bacterial activities. The results of this study concluded the potential uses of coumarin derived compounds, in medicine and drug discovery.

CHAPTER 1

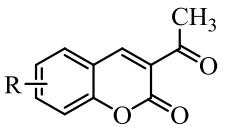
INTRODUCTION

1.1 Coumarin

Coumarin and many of its derivatives occur naturally. Coumarins owe their class name to 'Coumarou' which came from the plant *Coumarouna odorate*. Coumarin was first isolated in 1820 from the fruit of *Dipteryx odorata* Wild by Vogel (Bruneton, 1999). Coumarin is classified as a member of the benzopyrone family, which consist of a benzene ring fused with α-pyrone ring (**1**), known as1,2-benzopyrone. Benzopyrones can be further subdivided into benzo-α-pyrones, a class to which the coumarins belong and benzo-γ-pyrones, a class to which flavonoids are the principal members. The systematic name of coumarin is 2*H*-1-benzopyran-2-one.



Coumarin **(1)**



Substituted 3-acetyl coumarins **(2)**
R = Alkyl, alkoxy, halogen or any other substituent

A literature survey revealed that heterocyclic compounds containing coumarin shows diverse biological activities such as anticonvulsant (Dimmock et al., 2000), antioxidant (Osman et al., 2012), anti-HIV (Hariprasad et al., 1998), antibacterial (Arshad et al., 2011), anticoagulant (Jain et al., 2012), anti-cancer (Federica et al., 2010) and anti-fungal (Singh et al., 2010). Coumarin also has paramedicinal applications and it is used in the treatment of psoriasis and other dermatological disorders. Therefore, natural, semi-synthetic and synthetic coumarins are of immense value in the field of pharmaceutical research.

Coumarin is also widely used as an aroma chemical because of its specific odor, stability to alkali and temperature, tenacity and relatively cheap price. It has been used as a sweetener and fixative in perfumes, a fragrance enhancer for natural essential oils, blender in soaps and detergents, an aroma enhancer in tobacco and for imparting pleasant odors to industrial products. Their derivatives are used as intermediates for the synthesis of heterocyclic ring systems which are of great importance and are associated with a wide spread popularity in the field of medicine.

For example, the 3-acetyl coumarin derivatives (**2**), which were synthesized by the reaction of different substituted salicylaldehyde with ethylacetoacetate in the presence of piperidine, had been proved to be important.

1.2. Problem statement

Microbial infections are one of the leading causes for the suffering and death of human beings, among which tuberculosis is considered to be one of the most life threatening disease. It is caused by *Mycobacterium tubercule,* a very pathogenic bacterium. Despite lot of advances made in drug research, TB still remains one of the major health problems today. It is estimated that more than 2 billion people are infected with TB, thus leading to approximately 6% of all deaths worldwide (Lonnorth & Rviglione, 2008). Poverty, HIV and drug resistance are the major contributors to the resurging global TB epidemics (Cobert et al., 2006). TB is currently the leading killer of youth, women and AIDS patients in the world.

The global increase in resistance to antimicrobial drugs, including the antibacterial drugs, has also created several public health issues. The resistance development is a problem whenever antibacterial drugs are used for the treatment of bacterial infections. This is increasing high-risk in the populations who frequently require antibacterial therapy. Therefore, a renewed effort to increase awareness for

antibacterial drugs is needed in order to slow its development. In recent years, antibiotic-resistant microbial infections have become a growing concern. With the threat of traditional antibiotics and antimicrobial agents becoming obsolete, the development of new classes of antibiotics has become an important subject of current research. Naturally occurring defense methods have shown potential as alternatives to the currently available antibiotics, but bioavailability complications and the high cost of production have limited their translation into the clinics. Hence, there seems to be an urgent need for new anti-TB and antibacterial drugs. Arshad et al. (2011) recently synthesized a series of anti-TB and antibacterial coumarin derivatives. These compounds are potent enough to inhibit TB and bacterial infections.

These information prompted us also, to synthesize some new coumarin derivatives with possible anti-TB and antibacterial activity. Stability of coumarin in aqueous solution and heat resistant nature, make these compounds pharmacologically important.

1.3. Research objectives

Preparation of derivatives and evaluation of their activities is an interesting topic of research in the field of medicinal chemistry. The word derivative is a term of broad definition. It involves different kinds of chemical modifications of an existing drug by using different approaches, for preparing various structural combinations. Bearing this in mind, we set out to prepare several new series of biologically active compounds containing, coumarin, thiazole ring, thiazolidinone ring, thiadiazole ring and phenylacrylohydrazide unit within the molecular framework.

The present work focuses on three main research objectives:

i. To synthesize six new series of heterocyclic compounds by incorporating thiazole ring, thiazolidine ring, thiadiazole ring and phenylacrylohydrazide

moiety into coumarin core.

ii. To characterize all the synthesized compounds by different spectroscopic techniques (IR, 1D & 2D NMR, LC-MS), CHN elemental analysis and to determine and confirm the exact configuration of some derivatives by X-ray crystallography.

iii. To evaluate the antibacterial and anti-tuberculosis activities of the synthesized compounds against Gram-positive (*S. aureus* and *S. Pneumonia*) and Gram-negative (*E. coli*, *S. typhi* and *A. aerogenes*) bacterial strains and *Mycobacterium tuberculosis*, respectively.

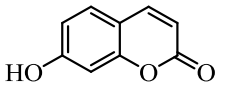
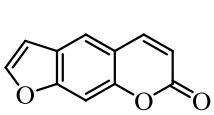
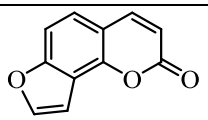
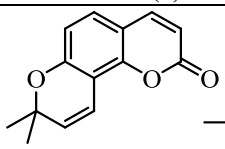
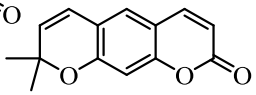
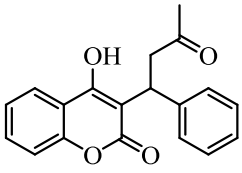
CHAPTER 2

LITERATURE REVIEW

2.1. Classification of coumarin

Gottlieb et al. (1978) used a biogenetic way which was based on the presence of number of nuclear oxygen atoms for the classification of coumarins. Many natural coumarins have also been classified by botanically derived names, mostly ending with the suffixes, "–ol" or "–one". Kennedy & Lacy., (2004) on the other hand, classified coumarin into four subgroups. By virtue of its structural simplicity coumarin has been assigned as the representative member of the benzo-α-pyrones (**Table 1.1**).

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

Classification	Features	Examples
Simple coumarins	Hydroxylated, alkoxylated or alkylated on benzene ring	 7-hydroxycoumarin **(3)**
Furanocoumarins	5-membered furan ring attached to benzene ring. Linear or Angular	 Psoralen **(4)**  Angelicin **(5)**
Pyranocoumarins	6-membered pyran ring attached to benzene ring. Linear or Angular	 Seselin **(6)**  Xanthyletin **(7)**
Pyrone-substituted coumarins	Substitution on pyrone ring, often at 3-C or 4-C positions	 Warfarin **(8)**

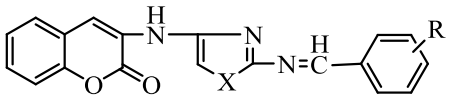
2.2. Biological activities of coumarin and its derivatives

Coumarins belong to a biologically active class of compounds as they show quite diverse biological activities. They have been reported to be wonder compounds possessing anticoagulant, antiallergic, vascodilatory, anthelmintic, diuretic, insecticidal, anticancer, antioxidant, anti tuberculosis, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antitumor and antibiotic activities. Coumarin derivatives are important pharmacological compounds and precursors in medicinal drug synthesis such as sulfathiazole, an antibiotic. These activities make coumarin compounds, attractive backbone derivatives for screening as new therapeutic agents.

2.2.1. Antibacterial activity of coumarin derivatives

During the past two decades, the developed immunity in the growing population of patients, with malignancies and transplant recipients, has resulted in an increase in severe opportunistic microbial infections. Therefore, the health problems arising, demands urgency, to develop new authentically genuine, low-toxic antimicrobial drugs, which can overcome the current medications.

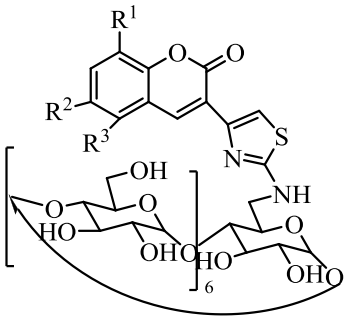
Therefore, in an attempt to find new agents to fight against bacterial infections, Singh et al., (2010) reported two series of coumarin based antibacterial compounds.They synthesized 3-[(2'-substituted benzylidene amino thiazol-4'-yl) amino] coumarins (**9a-d**) and 3-[(2'-substituted benzylidine amino oxazole-4'-yl) amino coumarins (**10a-d**). All the synthesized compounds were screened for their *in vitro* antibacterial activities. The results showed, that compound **9d** and **10d**, with *para* methoxy substituent had good antibacterial activity as compared to their homologues **9a-c** and **10a-c**.



9a-d: X= S
10a-d: X= O

Compound	R	Compound	R
9a	H	**10a**	H
9b	*p*-OH, *m*-OCH_3	**10b**	*p*-OH, *m*-OCH_3
9c	*o*-OH	**10c**	*o*-OH
9d	*p*-OCH_3	**10d**	*p*-OCH_3

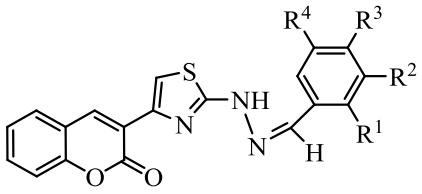
Kamal et al. (2010) had synthesized a new series of cyclodextrin linked coumarins (**11a-g**) and evaluated their antimicrobial activities. All the compounds showed significant antimicrobial activities against Gram-positive *viz. Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram-negative *viz. Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains. The compounds exhibited up to four fold improved biological activities as compared to their precursors.



11a-g

Compound	R^1	R^2	R^3
11a	H	H	H
11b	OCH_3	H	H
11c	H	Cl	H
11d	H	$CHCH_2CH_2CH$	$CHCH_2CH_2CH$
11e	Cl	Cl	H
11f	H	Br	H
11g	Br	Br	H

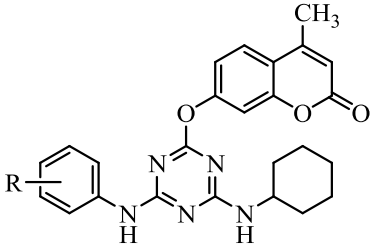
In addition to these, hydrazinyl thiazolyl coumarin derivatives (**12a-d**) were tested *in vitro* by Arshad et al., (2011) against different bacteria. Compound **12a**, having a hydroxyl group at *ortho* position, exhibited significant antibacterial activity.



12a-d

Compound	R^1	R^2	R^3	R^4
12a	OH	H	H	H
12b	H	OH	H	H
12c	H	H	OH	H
12d	OH	H	OH	H

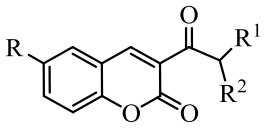
Moreover, very recently Parmar et al., (2013) tested the *in vitro* antibacterial activities of a new series of 2-(4-methyl-7-hydroxy-coumarin)-4-(cyclohexylamino)-6-(arylamino)-s-triazine coumarin derivatives (**13a-j**). All the compounds showed significant antibacterial activities against Gram-positive *viz. Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative *viz. Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains.



13a-j

Compound	R	Compound	R
13a	*p*-toludine	**13f**	*p*-chloroaniline
13b	*p*-fluoroaniline	**13g**	*m*-toluidine
13c	3-chloro-4-fluoroaniline	**13h**	(4-aminophenyl)(methyl) amide
13d	*p*-nitroaniline	**13i**	*m*-chloroaniline
13e	*p*-methoxyaniline	**13j**	*m*-bromoaniline

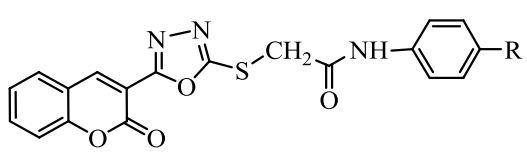
Kasumbwe et al. (2014) had reported *in vitro* antibacterial activities of coumarin based halogen compounds (**14a-f**). The result showed that compounds **14c** and **14f** exhibited very good antibacterial activities with MIC value of 0.75 mg/mL as compared to its analogues.



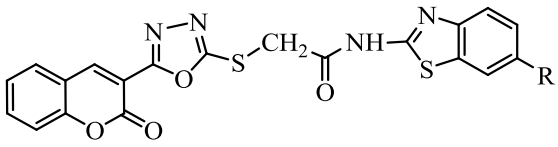
14a-f

Compound	R	R^1	R^2	Compound	R	R^1	R^2
14a	Br	H	H	**14d**	Cl	Br	H
14b	Cl	H	H	**14e**	Br	Br	H
14c	H	Br	H	**14f**	H	Br	Br

Patel et al. (2014) had synthesized two new series of coumarin-based oxadiazoles (**15a-k; 15-v**) and evaluated their *in vitro* antibacterial potential against several bacteria. There studies was based on structural relationship which showed, that up to what extent the presence of various electron withdrawing or electron donating groups, which were present on phenyl or benzothiazole ring system, can affect the activity profiles of the newer molecules. From the results it was concluded, that almost all the coumarin derivatives were found to show good MIC values ranging in between 6.25-100 μg/mL. However, exceptionally good activities were shown by the analogues, with electron donating groups such as halogen, methoxy and cyano substituents, present on phenyl or benzothiazole ring. Whereas, compounds with electron withdrawing groups such as the nitro group (**15f, 15q**), were found to show comparatively higher MIC values.



15a-k



15l-v

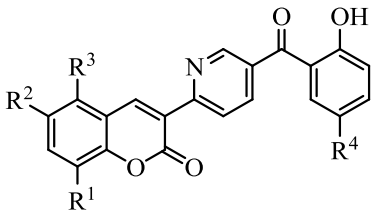
Compound	R	Compound	R
15a;15l	H	**15g;15r**	CN
15b;15m	Cl	**15h;15s**	CH_3
15c;15n	Br	**15i;15t**	OCH_3
15d;15o	F	**15j;15u**	OC_2H_5
15e;15p	I	**15k;15v**	$NHCOCH_3$
15f;15q	NO_2	**-**	-

2.2.2. Anti-tuberculosis activity of coumarin derivatives

Mycobacterium tuberculosis, which is responsible for most of the tuberculosis cases, is one of the most pathogenic bacterial strains ever reported. Tuberculosis has now become more severe, due to the resistance developed by *Mycobacterium tuberculosis*, against the first line as well as second line drugs. Multidrug-resistant TB cases which were reported in 2010, were in their highest level (Koul et al., 2011). In Malaysia, TB claimed some, 1,700 lives and infected 19,250 people in 2011 (WHO, 2011). Almost 90 % of the TB cases were patients with known HIV status, because activation of TB occurs when the person's immune system is somehow impaired, either by age, malnutrition, or by other diseases such as acquired immune-deficiency syndrome (AIDS). The cause of death due to TB was largely due to its late detection. Hence, there is urgent need to develop new molecules against this deadly pathogen. Coumarin and its derivatives have gained great therapeutic importance in the field of medicinal chemistry, as they display a fascinating array of pharmacological properties.

In this regard Bhila et al., (2013) had studied the anti-tuberculosis activities of pyridyl coumarin derivatives (**16a-l**), against *Mycobacterium tuberculosis* H37Rv.

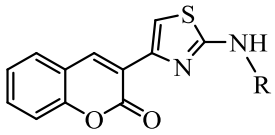
The results showed that all the coumarin compounds exhibited very good MIC values ranging in between 62.5-1000 μg/mL.



16a-l

Compound	R^1	R^2	R^3	R^4	Compound	R^1	R^2	R^3	R^4
16a	H	H	H	H	**16g**	H	Br	H	H
16b	H	H	H	CH_3	**16h**	H	Br	H	CH_3
16c	H	H	H	Cl	**16i**	H	Br	H	Cl
16d	OCH_3	H	H	H	**16j**	H	benzo	H	H
16e	OCH_3	H	H	CH_3	**16k**	H	benzo	H	CH_3
16f	OCH_3	H	H	Cl	**16l**	H	benzo	H	Cl

More studies were conducted by Arshad et al., (2011) on thiazole coumarin derivatives. All the derivatives (**17a-d**), were screened *in vitro* against *Mycobacterium tuberculosis*. Compounds **17c** and **17d** exhibited very good activities with the MIC values17 and 15 μM, as compared to the standard drug Isoniazid.

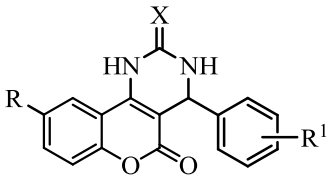


17a-d

Compound	R
17a	4-(1-iminoethyl)phenol
17b	diphenylmethanimine
17c	7-hydroxy-3(1-iminoethyl)-2*H*-chromen-2one
17d	6-bromo-3-(1-iminoethyl)-2*H*-chromen-2one

Very recently, Ambre et al., (2014) had synthesized a set of 16 coumarin based pyrimidine derivatives (**18a-p**) with anti-tubercular potential. They recorded the % inhibition of all the compounds at concentration level of 128 μM. The results

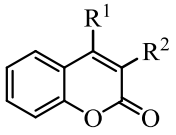
showed that the presence of electron donating groups like methyl, methoxy and hydroxyl on the phenyl ring system imparted greater anti-tubercular activity.



X=S; 18a-h
X=O; 18i-p

Compound	R	R^1
18a;18i	H	H
18b;15j	H	4-OCH_3
18c;15k	H	3,4-OCH_3
18d;18l	CH_3	4-OCH_3
18e;18m	CH_3	3,4-OCH_3
18f;18n	H	3-OCH_3;4-OH
18g;18o	H	3-Br
18h;18p	H	3-Br

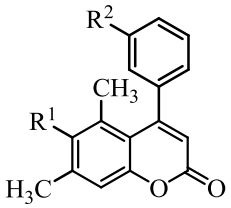
Moreover, Angelova et al., (2014) had synthesized eight coumarin-derived aminoalcohols and amidoamines (**19a-h**) and evaluated their *in vitro* anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Rv. Ethambutol was used as the standard drug. All of the tested compounds displayed, 10-20 times higher activities, ranging in between 0.32-19.2 μM as compared to the standard drug, which was found to display the MIC value of 7.22 μM.



19a-h

Compound	R^1	R^2
19a	$NH(CH_2)_2OH$	H
19b	$NHCH_2CH(OH)CH_3$	H
19c	$NH(CH_2)_2NHCOCH_3$	H
19d	$NH(CH_2)_3NHCOCH_3$	H
19e	$NH(CH_2)_2OH$	$N(CH_2)_2OH$
19f	$NH(CH_2)_3OH$	$N(CH_2)_3OH$
19g	$NHCH_2CH(OH)CH_3$	CHO
19h	C=O	$N(CH_2)_2OH$

In addition to this, Stanley at al., (2013) had synthesized, a very important diaryl coumarin derivatives series (**20a-d**), which kills *Mycobacterium tuberculosis*, by inhibiting fatty acid degradation protein D32 (FadD32). Fad32 is an essential enzyme which is required in the biosynthesis of mycolic acids. Mycolic acid is an important component of a typical *mycobacterium* cell-wall. These substituted coumarin inhibitors directly inhibit the acyl-acyl carrier protein synthetase activity of FadD32. As a result, they effectively block bacterial replication both *in vitro* and *in vivo*, in the same pathway as the established antibiotic Isoniazid.



20a-d

Compound	R^1	R^2
20a	pyridine	H
20b	phenyl methanol	H
20c	4-benzylmorphine	H
20d	4-benzylmorphine	NH_2

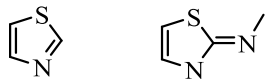
2.3. Synthesis of coumarin derivatives

Methods to synthesize coumarin derivatives have been well developed. Among which are, the Pechmann, Claisen, Perkin, Knoevenagel, Reformatsky, Hantzch and Wittig reactions.

2.3.1. Thiazolyl coumarins

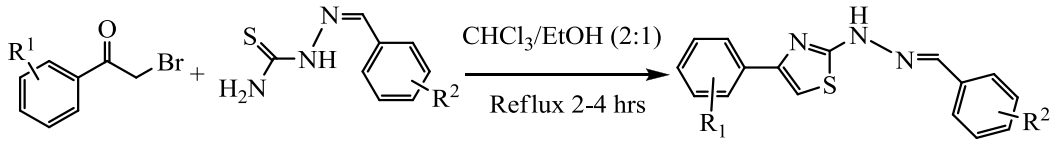
Thiazole or 1,3-thiazole is a five membered heterocyclic compound which contains nitrogen and sulphur atoms. The thiazole ring is planar and aromatic. Compounds containing thiazole ring had been reported to possess remarkable medicinal values such as antimicrobial (Singh et al., 2010), anti-oxidant (Osman et

al., 2012), anti-tuberculosis (Arshad et al., 2011) and anti-cancer (Belluti et al., 2010) properties.



Thiazole Thiazole-2-imine

Literature survey revealed that there are several methods to introduce thiazole ring to a coumarin nucleus. The most common method to synthesize thiazole is *via* Hantzch reaction, which was first discovered in 1887 by a German chemist, Hantzch. This reaction involves condensation of a compound bearing two hetero atoms on the same carbon atom (**Scheme1.1**) such as thioamide, thiourea, thiosemicarbazide, ammonium thiocarbamate, dithiocarbamate and their derivatives and a carbonyl compound with a halogen atom on the alpha carbon next to carbonyl group (Joule et al., 1995).

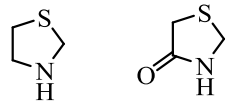


R[1]/R[2] = Alkyl, aryl or any other substituent
Scheme 1.1: Thiazole synthesis *via* Hantzch reaction

In the present study, Hantzsch cyclization was used to incorporate the thiazole ring into the coumarin nucleus as shown in **Scheme 1.1**. This method has several advantages as it allows alkyl, aryl or heterocyclic substituent to be placed in any position of the 5-membered ring. It involves simple work-up and usually a single product is obtained.

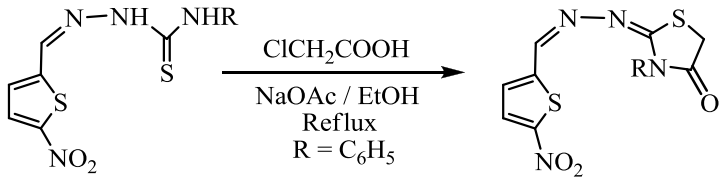
2.3.2. Thiazolidinone coumarins

Thiazolidine is a class of heterocyclic organic compounds with a 5-membered saturated ring with a thioether group and an amine group in 1 and 3 positions, respectively. Thiazolidine-4-one or thiazolidinone is one of its substituted forms. Compounds containing thiazolidinone ring had been reported to possess remarkable medicinal values such as antimicrobial (Lobo et al., 2012), anti-inflammatory (Rekha et al., 2011) and anticonvulsant (Amin et al., 2008).



Thiazolidine Thiazolidin-4-one

Thiazolidinone can be synthesized in several ways. For example, it can be synthesized in very good yield *via* the acetylation of thiosemicarbazone with sodium acetate and chloroacetic acid, in ethanol under reflux, following HSAB principle (Hard and soft acid and base) (Bouzroura et al., 2010) **(Scheme 1.2)**.

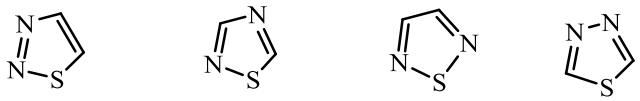


Scheme 1.2: Thiazolidinone synthesis *via* acetylation of thiosemicarbazone, using chloroacetic acid and sodium acetate

In the present work thiazolidinone ring system is introduced to the coumarin skeleton, accordingly to the method reported by Bouzroura et al., (2010) (S**cheme 1.2**). This method has its own importance as it allows alkyl, aryl or heterocyclic substituent to be placed in the third position of the 5-membered ring. It involves simple work-up and usually a single product is obtained.

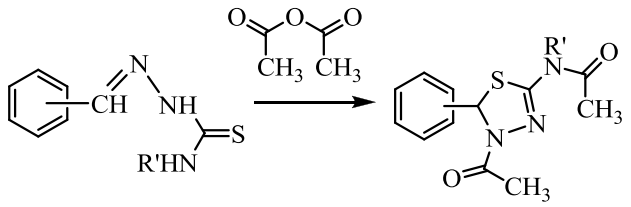
2.3.3. Thiadiazole coumarins

Thiadiazole is a five-membered heterocyclic compound having one sulphur and two nitrogen atoms. There are several isomers of thiadiazole, namely 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole and 1,3,4-thiadiazole. Compounds containing thiadiazole ring had been reported to possess remarkable medicinal values such as antimicrobial, antitumor, anti-tuberculosis and cytotoxic properties (El-Goharya et al., 2013).



1,2,3-thiadiazole 1,2,4-thiadiazole 1,2,5-thiadiazole 1,3,4-thiadiazole

A very convenient method to synthesize thiadiazole is *via* acetylation of thiosemicarbazone with acetic anhydride (**Scheme 1.3**), following HSAB principle. It is a cyclization reaction which involves addition of a mercapto group (thiol group or sufanyl group/SH) across CH=N bond and simultaneous acetylation of the NH group in the ring and NH_2 group in the side chain, thus resulting in the formation of 1,3,4-thiadiazole (Elokhina et al., 2002 &Mustafa et al., 2008).



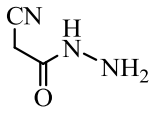
R' = H, CH_3

Scheme1.3: Thiadiazole synthesis *via* acetylation and cyclization of thiosemicarbazone, using acetic anhydride

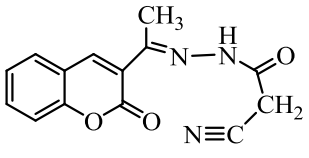
In the present work, the previously described method was used to introduce the thiadiazole ring to the coumarin core (**Scheme 1.3**). This reaction has advantage as the output is a single product, which can be purified by simple recrystallization.

2.3.4. Cyanoacetohydrazide coumarins

Cyanoacetohydrazide is a versatile intermediate in the synthesis of a variety of heterocyclic compounds and its behavior and mechanism of attack is similar to that of thiosemicarbazide. The β-functional nitrile moiety of this molecule is a favorable unit. Cycloaddition of this unit with numerous reagents will give different heterocyclic compounds of various ring sizes, with one or several heteroatoms. Cyanoacetohydrazide and its derivatives were known to possess versatile biological values and used as pharmaceutical ingredients (Elnagdi et al., 1991 and Gilman et al., 1985), herbicides (Cosales et al., 1998), antibacterial agents (Marinis et al., 1975) and dyes (Fahmy et al., 1980 & Fahmy et al., 1982). Cyanoacetohydrazide coumarins are compound in which the cyanoacetohydrazide is attached to the coumarin molecule at the position bearing the carbonyl group and hence, it is also known as coumarin cyanohydrazone.



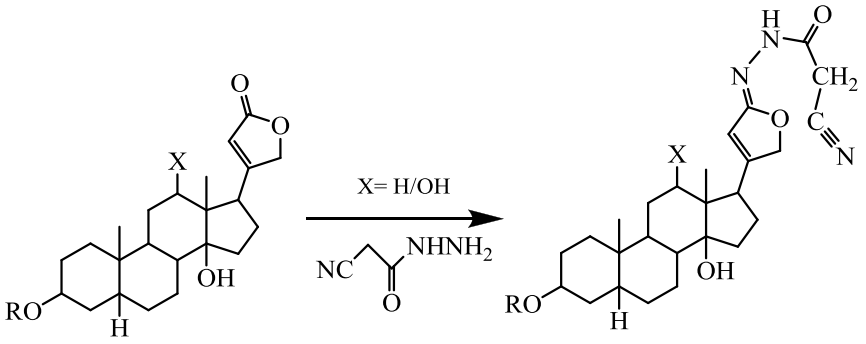
Cyanoacetohydrazide



Cyanoacetohydrazide coumarin
or Coumarin cyanohydrazone

An efficient way to synthesize cyanohydrazone derivative in good yield is *via* the reaction of cyanoacetohydrazide and ketone in methanol, in the presence of hydrochloric acid, in an ice bath **(Scheme 1.4)**. Coumarin cyanohydrazone is a schiff's base synthesis, as they are synthesized by the reaction between an amine and carbonyl compound. This method has been adopted in the present study, in order to incorporate the cyanoacetohydrazide moiety into the coumarin core. The reaction is very similar to the preparation of simple thiosemicarbazones. The precipitate

obtained *via* this reaction is filtered, washed by ethanol and recrystallized from methanol (Doss et al., 2001).

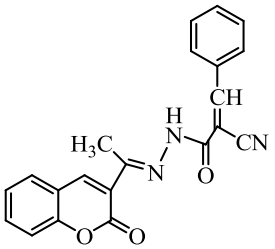


R/X= Alkyl, aryl or any other substituent
Scheme 1.4: Cyanohydrazone synthesis *via* Schiff"s base synthesis, using cyanoacetohydrazide and ketone

In the present work, the previously described method was used to introduce the cyanoacetohydrazide unit to the coumarin core (**Scheme 1.4**). The advantage of this method is, single product in good yield.

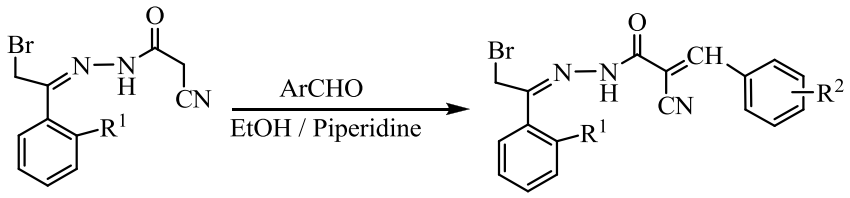
2.3.5. 2-Cyano-3-phenylacrylohydrazide coumarins

2-Cyano-3-phenylacrylohydrazide coumarins are compounds in which one of the methylene protons of cyanohydrazone is replaced by aromatic aldehydes *via* elimination of a water molecule. 2-Cyano-3-phenylacrylohydrazide derivatives are very important pharmacological group of compounds possessing antimicrobial (Mohareb et al., 2007), antifungal (Mohareb et al., 2007), antitumor (Mohareb et al., 2010) and many other important medicinal properties.



2-Cyano-3-phenylacrylohydrazide coumarin

An efficient way to synthesize 2-cyano-3-phenylacrylohydrazide compounds is by Claisen Schmidt condensation reaction. The reaction involves condensation of aldehyde or ketone, with the carbonyl compounds lacking an alpha hydrogen, in the presence of acid or base as a catalyst. After the reaction completes, the reaction mixture is poured in ice cold water, containing few drops of HCl (Mohareb et al., 2010). The solid product thus obtained, is collected and recrystallized to afford pure 2-cyano-3-phenylacrylohydrazide compounds **(Scheme 1.5)**.



$\mathbf{R^1/R^2}$ = Cl, Br, NO_2 or any other substituent

Scheme1.5: 2-Cyano-3-phenylacrylohydrazide derivative synthesis *via* Claisen Schmidt condensation reaction between aromatic aldehydes and cyanoacetohydrazide derivative.

In the present study, 2-cyano-3-phenylacrylohydrazide coumarin was synthesized according to the method reported by Mohareb et al., (2010) (**Scheme 1.5**). This reaction has advantage as it involves simple work-up and usually a single product is obtained.

CHAPTER 3

MATERIALS AND METHODS

3.1. Chemicals and solvents

The commercial chemicals and reagents used in the syntheses were either of analytical reagent grade, laboratory reagent grade or HPLC grade. All solvents were used without further purification, unless otherwise stated (**Table 3.1**).

Table 3.1: Chemicals, reagents and solvents.

Chemicals, Reagents, Solvents	Grade
Acetic acid glacial	AR grade, QRëC, Malaysia
Acetone	AR grade, QRëC, Malaysia
Ammonium hydroxide, 28-30% NH_3	AR grade, Sigma-Aldrich,
Acetic anhydride	Sigma-Aldrich, USA
Bromine	Merck, Germany
Benzaldehyde	Acros Organics, Belgium
5-Bromo-2-hydroxybenzaldehyde	Acros Organics, Belgium
4-Bromobenzaldehyde	Acros Organics, Belgium
2-Bromoacetophenone(phenacyl bromide)	AR grade, Sigma-Aldrich,
2-Bromophenyl thiourea	AR grade, Sigma-Aldrich,
Calcium chloride, anhydrous, granular	R & M Chemical Essex, UK
4-Chlorobenzaldehyde	Acros Organics, Belgium
Chloroform	AR grade, QRëC, Malaysia
Chloro acetic acid	Sigma-Aldrich, USA
Cyano acetic acid hydrazide	Sigma-Aldrich, USA
Dichloromethane	AR grade, QRëC, Malaysia
Diethyl ether	AR grade, QRëC, Malaysia
Dimethyl sulfoxide-d_6 99.8 atom % D	Sigma-Aldrich, USA
Dioxane	AR grade, QRëC, Malaysia
2,4-Dihydroxybenzaldehyde	Acros Organics, Belgium
3,4-Dichlorophenyl thiourea	Sigma-Aldrich, USA
Ethanol, 99.7%	AR grade, QRëC, Malaysia
Ethyl acetate	AR grade, Fisher Scientific,
Ether	AR grade, QRëC, Malaysia
Ethyl acetoacetate	Sigma-Aldrich, USA
2-Hydroxybenzaldehyde	Acros Organics, Belgium
2-Hydroxy-4-methoxy benzaldehyde	Sigma-Aldrich, USA
2-Hydroxy-5-methoxy benzaldehyde	Sigma-Aldrich, USA
2-Hydroxy-6-methoxy benzaldehyde	Sigma-Aldrich, USA
2-Hydroxy-5-nitro benzaldehyde	Sigma-Aldrich, USA

4-Hydroxybenzaldehyde	Acros Organics, Belgium
Hydrochloric acid	R & M Chemical Essex, UK
Methanol	AR grade, QRëC, Malaysia
2-Methoxyphenyl thiourea	Sigma-Aldrich, USA
4-Methoxyphenacyl bromide	Sigma-Aldrich, USA
4-Methylphenacyl bromide	Sigma-Aldrich, USA
Molecular sieves type 4A, beads	Fluka, Switzerland
N-Ethyl thiourea	Sigma-Aldrich, USA
N-Methyl thiourea	Sigma-Aldrich, USA
N,N–Diethyl thiourea	Sigma-Aldrich, USA
N,N–Dimethyl thiourea	Sigma-Aldrich, USA
N,N–Diphenyl thiourea	Sigma-Aldrich, USA
Piperidine	BDH, England
Potassium bromide, FT-IR grade	Sigma-Aldrich, USA
Salicylaldehyde	Sigma-Aldrich, USA
Sodium acetate anhydrous	Systerm, Malaysia
TLC silica gel 60 F254, aluminium sheets,	Merck, Germany
Thiosemicarbazide, 99%	Sigma-Aldrich, USA

3.1.1. Preparation of ethanol free chloroform

Ethanol free chloroform was prepared by adding concentrated sulphuric acid to chloroform and the solution was gently shaken in a separating funnel. The acid layer was discarded and chloroform was washed with distilled water, until the washing becomes neutral in pH. The chloroform was then dried overnight, over anhydrous calcium chloride and distilled the following day. Chloroform purified with this method contains less than 0.001% (w/w) of ethanol (Williamson et al., 1995).

3.1.2. Preparation of dry ethanol

Dry ethanol, was prepared according to the method reported by Armarego & Chai, (2009) with slight modifications. Ethanol was dried by refluxing it in anhydrous calcium oxide (250 gm/L) for 6 hours. The mixture was then allowed to stand overnight and filtered the following day.

3.1.3. Reaction monitoring

The progress of all reactions was monitored by thin layer chromatography (TLC). The reaction mixture and the starting materials were both spotted on TLC plates, using different solvent systems like petroleum:ether and petroleum:ethylacetate. The appearance and disappearance of product and reactant spots respectively on TLC, determined the optimum reaction completion time for all the reactions. Spots developed on the TLC plates were visualised under UV lamp either at 254 and 365 nm of wavelength and in some cases using an iodine chamber.

3.1.4. Moisture-sensitive reactions

All moisture sensitive reactions were performed at normal atmospheric pressure in ice bath between 0-5^{o}C. A calcium chloride guard tube was always affixed to the reaction flask in order to absorb any moisture during the reactions. All glassware used in the reactions were dried overnight in an oven at 120^{o}C. Solvents used for the reactions were distilled and dried before use.

3.1.5. Recrystallization

All crude products were purified by recrystallization. Single-solvent and multi-solvent systems were used for the purification of crude products. The crude products were dissolved in hot condition either in a single solvent or mixture of solvents depending upon their solubility. Usually a solvent which dissolves the crude products sparingly at room temperature is suitable for recrystallization in hot condition. The hot clear solution of the crude product was filtered and concentrated by heating to half of its original volume and then allowed to cool slowly at room temperature as the slow cooling process gave bigger crystals. The crystals obtained

were filtered by suction using a Hirsch funnel and washed with suitable solvents like ether or ethanol.

3.1.6. Melting point measurement

Melting points of all the synthesized compounds were determined by Stuart Scientific SMP-1 (UK) melting point apparatus in a temperature range of 25–350 °C.

3.2. Characterization of compounds

All the synthesized compounds were characterized by different spectroscopic techniques including Infrared (IR) and 1D Nuclear Magnetic Resonance (NMR) spectroscopy. However, new derivatives were additionally characterized by LC-MS, 2D Nuclear Magnetic Resonance (NMR) spectroscopy, elemental analysis (CHN) and X-Ray Crystallography techniques. All the spectroscopic analyses and MP measurements were carried out at the School of Chemical Sciences, USM whereas the X-Ray Crystallographic analysis was conducted at the School of Physics, USM.

3.2.1. Liquid Chromatography Mass Spectrometry (LC-MS)

The molecular mass of the synthesized compounds was determined by LC-MS. All the measurements were conducted on an Agilent Technologies 6224 TOF LC/MS spectrometer at the Department of Chemistry, USM. Agilent Mass Hunter software was used to process all the spectra obtained. Samples were prepared as solutions of 0.1 to 1.0 μg/mL in HPLC grade acetonitrile. The measurements were carried out in the positive mode.

3.2.2. Infrared (IR) Spectroscopy

IR spectra of all the synthesized compounds were recorded on Perkin-Elmer System 2000 FT-IR spectrometer (England, UK). The samples were prepared as

potassium bromide (KBr) discs and scanned in the measurement range of 400- 4000 cm^{-1}. All the data was analysed by Perkin-Elmer software.

3.2.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance spectra of all the synthesized compounds were recorded on a Bruker Avance 500 spectrometer operated at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, respectively. The 2D spectra (COSY, HMQC and HMBC) of the new compounds were recorded on a Bruker Avance 500 spectrometer. The spectra were recorded in deuterated solvents (chloroform-d_1 or dimethyl sulfoxide-d_6) containing tetramethylsilane (TMS) as the internal standard. Chemical shifts δ, were measured with reference to the TMS signal at 0.00 ppm and the coupling constants J, were reported in hertz (Hz).

3.2.4. Elemental Analysis

Elemental analyses for all the synthesized new compounds were performed on a Perkin Elmer series II, 2400 CHN analyzer.

3.2.5. X-Ray Crystallography Analysis

Single crystal X-ray crystallographic data analysis was performed on a Bruker SMART APEXII CCD area-detector diffractometer and the crystallographic structures were solved and refined by using SHELXTL and MERCURY software.