SYNTHESIS, CHARACTERIZATION AND ANTI-MYCOBACTERIAL ACTIVITY OF NEW 1,3,4-OXADIAZOLE DERIVATIVES

NURUL SYAZANA BINTI HASMARUDDIN

UNIVERSITI SAINS MALAYSIA

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by

NURUL SYAZANA BINTI HASMARUDDIN

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In the Name of Allah S.W.T, the Most Gracious and the Most Merciful

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LIST OF SYMBOLS AND ABBREVIATIONS

1D NMR	One Dimensional Nuclear Magnetic Resonance
2D NMR	Two Dimensional Nuclear Magnetic Resonance
DCM	Dichloromethane
DEPT NMR	Distortionless Enhancement by Polarization Transfer
FT-IR	Fourier Transformer Infrared
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{1}H-^{1}H COSY	Correlation Spectroscopy
1H-^{13}C HSQC	Heteronuclear Single Quantum Correlation
^{1}H-^{13}C HMBC	Heteronuclear Multiple Bond Correlation
MTT	(3-(4,5-Dimethyl-1-2thizolyl)-2,5-diphenyl-*2H*-tetrazolium bromide
TLC	Thin Layer Chromatography
TB	Tuberculosis
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
$CDCl_3$	Deuterated Chloroform
DMSO-d_6	Deuterated Dimethyl sulfoxide
s	Singlet
d	Doublet
dd	Doublet of doublets
m	Multiplet
t	Triplet
Hz	Hertz
mHz	Megahertz
µg/mL	Microgram per mililitre

μM	Micromolar
m.p	Melting point
δ	Chemical shift
ppm	Parts per million
μg	Microgram
°C	Degree Celsius
min	Minute
h	Hour

SINTESIS, PENCIRIAN DAN AKTIVITI ANTI-MIKOBAKTERIA TERHADAP SEBATIAN BARU 1,3,4-OKSADIAZOL

ABSTRAK

Dalam kajian ini, 20 terbitan baharu 2-alkilbenzisulfanil-5-tertukarganti-1,3,4-oksadiazol (**5**a-**j** dan **6a-j**) telah disintesis dalam empat langkah tindak balas daripada asid karboksilik sebagai bahan permulaan dan telah menghasilkan (40-92%) sebatian. Sebatian telah disintesis dengan pelbagai penukar ganti termasuk sebatian dengan penderma elektron serta kumpulan penarik elektron. Semua sebatian telah dicirikan menggunakan analisis unsur Inframerah Jelmaan Fourier (FT-IR) dan Resonan Magnetik Nuklear (NMR) dan juga analisa unsur karbon, nitrogen dan hidrogen. Beberapa sebatian telah dipilih untuk ujian anti-mikobakteria terhadap organisma *M. smegmatis* dan *M. tuberkulosis* H3Ra. Sebanyak empat belas sebatian menunjukkan aktiviti anti-mikobakteria terhadap *M. smegmatis* dengan nilai MIC dalam julat 25-1600 μg/mL. Nilai MIC dan MBC terhadap *M. smegmatis* menunjukkan bahawa hanya sebatian **5c** dan **5d** menunjukkan aktiviti anti-mikobakteria yang baik dengan nilai masing-masing 78 μM dan 68 μM. Sebahagian daripada sebatian menunjukkan aktiviti sederhana kepada lemah. Kajian interaksi sebatian **5c** dan **5d** dengan isoniazid terhadap *M. smegmatis* menggunakan kaedah *checkerboard* menghasilkan interaksi tambahan. Asai masa-pembunuhan pada gabungan sebatian **5c** dan **5d** dengan isoniazid terhadap *M. smegmatis* menghasilkan kadar pembunuhan yang lebih tinggi berbanding INH pada tempoh akhir kajian masing-masing iaitu 88.22 % dan 77.10 %. Oleh itu, sebatian **5c** dan **5d** menunjukkan potensi sebagai ubat anti-tuberkulosis.

SYNTHESIS, CHARACTERIZATION AND ANTI-MYCOBACTERIAL ACTIVITY OF NEW 1,3,4-OXADIAZOLE DERIVATIVES

ABSTRACT

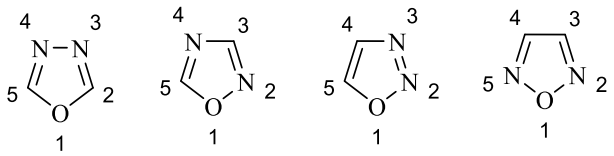
A total of 20 new 2-alkylbenzysulfanyl-5-substituted-1,3,4-oxadiazoles derivatives (**5a-j** and **6a-j**) were synthesized in four-step reaction pathways from carboxylic acid analogues as the starting materials, with moderate (40%) to excellent yields (92 %). These compounds continued a wide range of substituents including electron donating as well as electron-withdrawing groups. All these compounds were characterized using Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy and elemental analysis. Some of the synthesized compounds were assayed for anti-mycobacterial activity against surrogate tuberculosis organisms (*Mycobacterium smegmatis* and *Mycobacterium tuberculosis* H3Ra). Fourteen compounds exhibited inhibition against *M. smegmatis* with MIC values in the range of 25-1600 µg/mL. Results of anti-mycobacterial assay against *M. smegmatis* showed that only **5c** and **5d** had good inhibition with MIC and MBC values of 78 µM and 68 µM, respectively. Some of the compounds showed moderate to weak inhibition. Interaction study of the respective compounds **5c** and **5d** with isoniazid against *M. smegmatis* using the checkerboard method produced an additive interaction. The time-kill assay on the combination of compounds **5c** and **5d** with INH against *M. smegmatis* produced a higher killing rate compared to drug INH at the end of the period of study which are 88.22 % and 77.10 %, respectively. Therefore, compounds **5c** and **5d** are potential anti-tuberculosis drug candidates.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Research in the field of pharmaceutical organic chemistry deals with the discovery of new and safe therapeutic agents of clinical importance. Heterocyclic compounds constitute largest division of organic chemistry. The five-membered ring heterocyclic compounds continue to attract interest due to the wide range of the biological activities they exhibit (Kasyap *et al*., 2011). The chemistry of five membered heterocyclic compounds has been an interesting field of study for long time. In the past decade, most nitrogen containing heterocyclic systems have been used as a source to discover new compounds, for example, the oxadiazole derivatives. Oxadiazoles are a class of five-membered heterocyclic aromatic compounds of the azole family and they are derived from furan by replacing two carbon group with two pyridine-typed nitrogen. Particularly, oxadiazoles have been successfully tested against several diseases and therefore this class of compound received special attention in pharmaceutical chemistry due to diverse medicinal potential; fungicidal, bactericidal, anticancer, antitubercular activities, and et cetera (Mishra *et al*., 2010). The oxadiazoles exist in different isomeric forms such as 1,3,4-oxadiazole (**i**), 1,2,4-oxadiazole (**ii**), 1,2,3-oxadiazole (**iii**) and 1,2,5-oxadiazole (**iv**). They are classified according to the position of the nitrogen atoms in the ring.



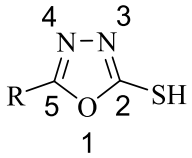
i **ii** **iii** **iv**

Isomers of Oxadiazole

1,3,4-oxadiazole (**i**) and 1,2,4-oxadiazole (**ii**) are better known and widely studied by researchers because of their many significant chemical and biological properties, while, the 1,2,3-isomer (**iii**) is unstable and ring-open to form the diazoketone tautomer (John *et al*., 2013). Among these compounds, **i** showed the highest studies and has increased considerably.

Substituted 1,3,4-oxadiazole derivatives display various types of biological activities including antibacterial (Loknatha *et al*., 1999; Mogilaiah *et al*., 2006 and Rubab *et al*., 2016), antimicrobial (Sahin *et al*., 2002 and Khalilullah, *et al*., 2011), anti-mycobacterial (Navarrete *et al*., 2007; Karabanovich *et al*., 2016; Zhou *et al*, 2017), antifungal (Li *et al*., 2006 and Chen *at el*., 2007), anti-inflammatory (Bhandari *et al*.,2008; Rathore *et al*.,2016) and anti-viral activities (Zhan and Liu, 2011). Among the substituted 1,3,4-oxadiazole derivatives; 5-substituted-1,3,4-oxadiazole-2-thiol (**v**) continuously draws interest for the development of new drug moieties and have increasing importance as compounds with biological activities (Mekuskeine *et al*., 2003). They received a great deal of attention in heterocyclic chemistry as versatile intermediates since the thiol group on oxadiazole ring undergoes nucleophilic substitution reactions readily (Horning *et al*., 1972 and Mekuskiene *et al*., 2003). In addition, their derivatives play an important role in the field of coordination chemistry because of their potential multifunctional donor sites, via either exocyclic sulfur or

endocyclic nitrogen atoms (Wang *et al*., 2007 and Singh *et al*., 2009) and with nucleophiles at the $C_{(2)}$ atom (Pancechowska *et al*, 1993 and Reid *et al*., 1976).



v

5-substituted-1,3,4-oxadiazole-2-thiol(thione)

Therefore, 1,3,4-oxadiazole (**i**) has become an important moiety for the development of new drugs (Oliveira *et al*., 2012). In view of these findings, an attempt has been made to synthesis 5-substituted-1,3,4-oxadiazole-2-thiol derivatives.

1.2 Problem Statement

Tuberculosis (TB) is an infectious respiratory disease caused by *Mycobacterium tuberculosis* (Centers for Disease Control and Prevention (CDC), 2016). This airborne disease is transmitted in droplets formed by coughing, talking or singing of an infected person, which affects the lungs of the people infected by it. TB has recently re-emerged and becomes one of the major public health problems worldwide. According to the World Health Organisation (WHO), approximately two million people worldwide died due to tuberculosis, and nine million was infected yearly (WHO, 2018). So far, the only vaccine against TB bacterial, Bacillus Calmette-Guerin (BCG) vaccine is unable to extend to adults, thus making susceptible people at higher risk of being infected (CDC, 2016). The rapid increase in multidrug-resistant TB (MDR-TB) supersedes other risk factors. MDR-TB was known to be resistant to at least isoniazid and rifampicin, two of the first-line anti-TB drugs (WHO, 2018). Schatz and Waksman, who first discovered streptomycin in 1944 was also reported to resist to this drug then

on 1960s a new drug, rifampicin was developed and became the new spotlight in developed countries. However, soon there were alerts in a few regions of the world especially in Russia and China as the MDR-TB was at critical level due to less surveillance of drug resistance (Espinal, 2003). The present re-emergence of MDR-TB makes the discovery of new drug as a priority to prevent the generation of new resistant mycobacterial strains (Koul *et al*., 2011).

The discovery and development of new drugs is a continuous process in the effort to combat *M. tuberculosis*. Many classes of old drugs that were discovered decades ago have now become new antibiotic candidates of chemically re-engineered molecules. The new drugs, which originally derived from the existing anti-bacterial drug classes had undergone remodelling in their chemical structures to improve their anti-bacterial strength. Hence, there seems to be an urgent need for new anti-TB drugs. Karabanovich *et al*. (2016) recently synthesized a series of anti-TB 5-substituted-1,3,4-oxadiazole-2-thiol derivatives. Since a large number of synthesized 5-substituted-1,3,4-oxadiazole-2-thiol derivatives have shown pharmacological potential as anti-TB agents, this research work focused on the modification of 1,3,4-oxadiazole scaffold into various bioactive compounds and the investigation of their antimycobacterial activity focusing on surrogate tuberculosis organisms.

1.3 Objectives

The main objectives of this study are:

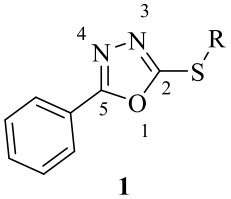
1. To synthesise two new series of 5-substituted-1,3,4-oxadiazole-2-thiol derivatives and characterise all the synthesised compounds by different spectroscopic techniques (FTIR, 1D & 2D NMR) analysis.
2. To investigate the anti-mycobacterial activity of the synthesised 5-substituted-1,3,4-oxadiazole-2-thiol derivatives against surrogate tuberculosis organisms (*Mycobacterium smegmatis* and *Mycobacterium tuberculosis* H3Ra).
3. To study the interaction of the most active 5-substituted-1,3,4-oxadiazole-2-thiol derivatives with first-line anti-tuberculosis drugs against the test organisms using a checkerboard assay.
4. To assess the killing rate of the most active 5-substituted-1,3,4-oxadiazole-2-thiol derivatives against the test organisms using a time-kill method.

CHAPTER 2

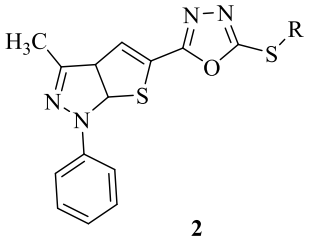
LITERATURE REVIEW

2.1 2,5-Substituted-1,3,4-oxadiazole

Substituted 1,3,4-oxadiazole derivatives have attracted much attention and display various types of biological activities. The synthesized compounds 2,5-subtituted-1,3,4-oxadiazoles exhibited varying antibacterial potential. Rubab *et al*., 2016 reported that a modification in the structure which changes the thiol position in the parent oxadiazole molecule by substituting different aryl/alkyl moieties may affect its antibacterial potential. Subsitution at position C-2 with an iso-propyl group, **1** showed inhibition potential against *S. aureus*.



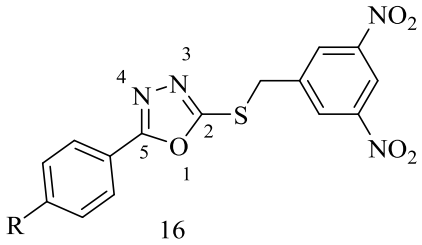
1



2

Some new 5-(3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazol-5-yl)-1,3,4-oxadiazole-2-thiol derivatives (**2**) have been found to possess considerable anti-inflammatory property. In 2015, Mahajan and co-workers prepared similar compounds with the same core moieties and these compounds were screened for their anti-inflammatory activity. They had successfully synthesized thiophene-fused pyrazole derivatives incorporated 1,3,4-oxadiazoles-2-substituted-thiols **2** and studied their anti-inflammatory activity using the HRBC membrane stabilization method with

Diclofenac sodium as the standard drug. In addition, Mahajan reported that the incorporation of a sulfur linkage had resulted in enhanced activities of the compounds.



16

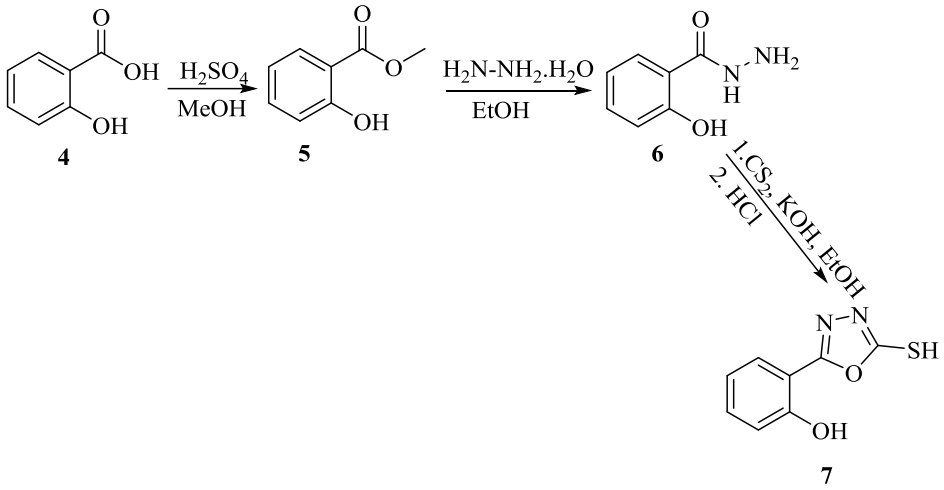
R= -OCH_3, Cl, NO_2
3a **3b** **3c**

Another research project done by Karabanovich *et al*., (2016), the development candidates of anti-mycobacterial agents had using compounds **(3a-c)** as the lead molecules with excellent potency against drug-susceptible and multidrug-resistant *M. tuberculosis*, without cross resistance with first- and second-line anti-TB drugs. The MIC value of the most active compound was as low as 0.03 μM (approximately 0.011 μg/mL). Futhermore, these compounds exhibited excellent activity against the non-replicating *M.tuberculosis*. strain SS18b-Lux. They determined that 3,5-dinitro substitution has a crucial role in antimycobacterial activity. Thus, any changes in the position or number of nitro groups led to a significant decrease in antimycobacterial activity.

2.2 Synthesis of 5-substituted-1,3,4-oxadiazole-2-thiol

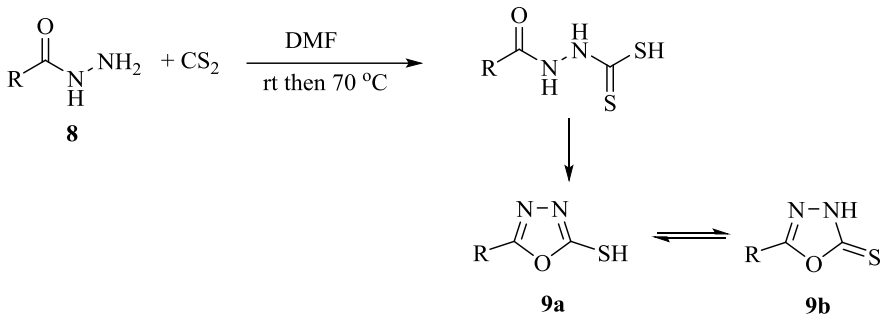
The synthesis and evaluation of biological activities of 1,3,4-oxadiazole and their derivatives were given attention after the discovery of the first 1,3,4-oxadiazole compound. Several synthesis methods of 1,3,4-oxadiazole and its derivatives have been reported in the literature. 1,3,4-Oxadiazole a liquid was first prepared by Ainsworth in year 1965 by the thermolysis of ethylformate and hydrazine, at atmospheric pressure.

Initially, a methyl salicylate (**5**) was synthesized by refluxing the 2-hydroxyl-benzoic acid (**4**) in methanol in the presence of sulfuric acid as catalyst. Then, the hydrazide (**6**) was obtained by heating the ester with hydrazine hydrate. Lastly for the preparation the oxadiazole (**7**), the hydrazide was heated with tan alcoholic solution of KOH and CS_2 under reflux followed by acidification with HCl to give the titled product that was reported to show promising antibacterial activity (Khiati *et al*., 2007) Scheme 2.1.



Scheme 2.1: Synthesis of 5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2-thiol.

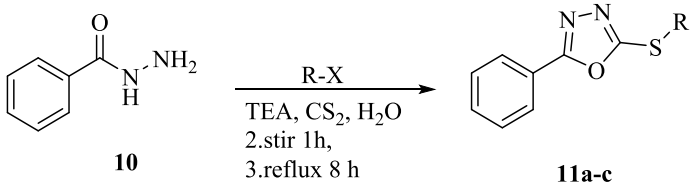
A research project was initiated by Soleiman-Beigi *et al*., (2013) in search for 5-subsituted-1,3,4-oxadiazole-2-thiol using dimethylformamide (DMF) as solvent. It was reported that the use of DMF solvent has a great effect on the yield and this solvent is miscible in the water with save to environment. A mixture of hydrazide and carbon disulphide in DMF was stirred for 15 min at room temperature then heated at 70 °C for 3-12 hours until the ring closure was completed Scheme 2.2. In addition, DMF was found to be the most convenient medium to facilitate the synthesis of 5-subsituted-1,3,4-oxadiazole-2-thiol which assisted the formation of tautomer **9a**.



Scheme 2.2: 5-Subsituted-1,3,4-oxadiazole-2-thiol, **9a**

2.3 Synthesis of 2-alkylbenzysulfanyl-5-substituted-1,3,4-oxadiazoles derivatives

Several methods were reported in the literature for the synthesis of 2-alkylbenzysulfanyl-5-substituted-1,3,4-oxadiazoles. Aryanasab and co-workers (2011) employed a one-pot synthesis by adding the acid hydrazide (**10**) with CS_2 in water. The reaction mixture was stirred at room temperature for 1 hour then alkyl halide was added and it was stirred for 6 hours. The mixture was then refluxed 90 °C in an oil bath for 8 hours, and after completion, the mixture was cooled to room temperature. The reaction was illustrated in Scheme 2.3.

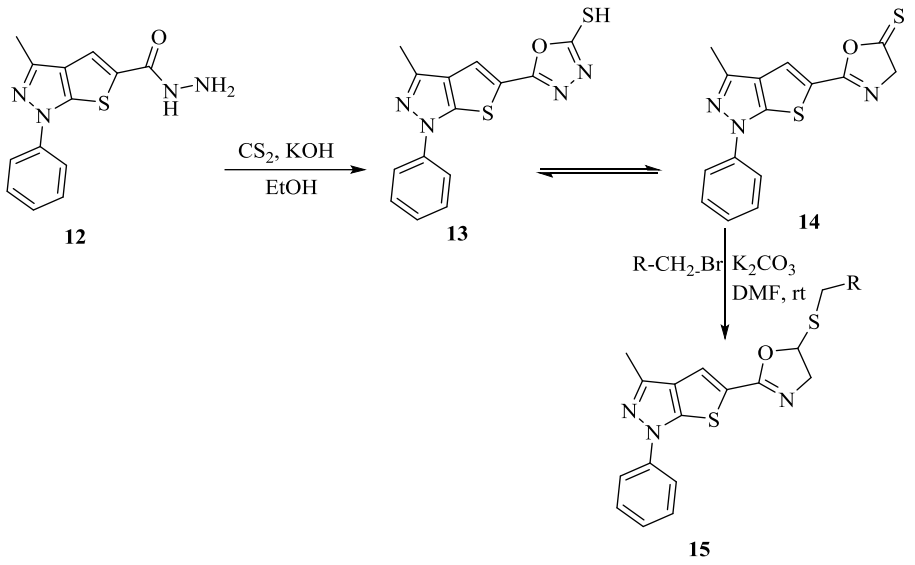


a: R=-CH_2CH_3
b: R=-CH_2-C_6H_5
c: R=-$CH_2CH_2CH_2CH_3$

Scheme 2.3: One-pot synthesis of 1,3,4-oxadiazoles (**11a-c**) in water.

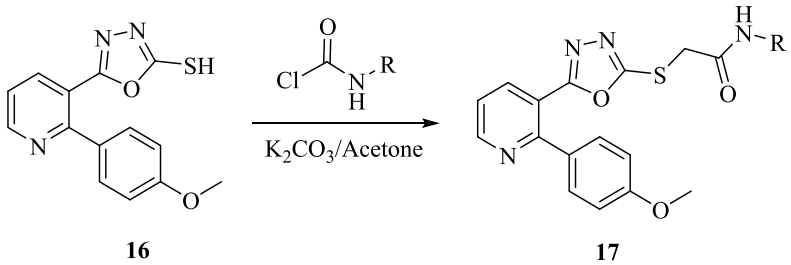
As reported by Mahajan and co-worker, 5-(3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazol-5-yl)-1,3,4-oxadiazole-2-thiol derivatives (**15**) were synthesised by adding the alkyl/aryl moieties in the presence of potassium carbonate with 65-79 % yield .

The reaction mixture was stirred at room temperature overnight as illustrated in Scheme 2.4.



Scheme 2.4: Synthesis of 5-(3-methyl-1-phenyl-1*H*-thieno[2,3-*c*] pyrazol-5-yl)-1,3,4-oxadiazole-2-thiol derivatives (**15**)

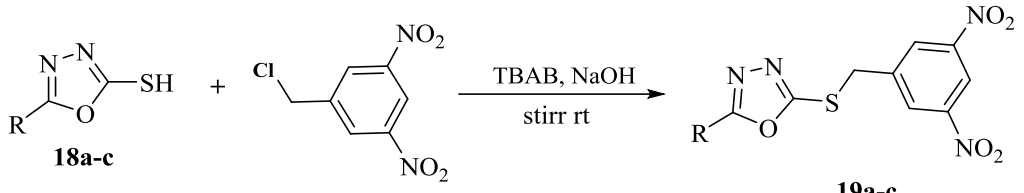
In 2014, Vinayak and co-workers has established a linear synthetic pathway to synthesise of derivatives (**17)** by reacting different acetamides which is 2-chloro-N-(arylsubstituted) acetamides with 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol (**16**) in the presence of K_2CO_3 and acetone (Scheme 2.5).



Scheme 2.5: Linear synthesis of 2-chloro-(N-aryl substituted) acetamide derivatives.

Karabanovich *et al*. (2016) have synthesized 5-alkyl/Aryl-2-(3,5-dinitrobenzylsulfanyl)-1,3,4-oxadiazoles (**19a-c**) which showed good anti-mycobacterial activities. These compounds **(19a-c**) was synthesized in the presence of

tetrabutylammonium bromide (TBAB) as a phase-transfer catalyst. The reacion was illustrated in Scheme 2.6. Target compounds (**19a-c**) were obtained in 76-83 % yield.



a:R = -OCH_3
b:R= -Cl
c:R= -NO_2

Scheme 2.6: Synthesis of 5-alkyl/Aryl-2-(3,5-dinitrobenzylsulfanyl)-1,3,4-oxadiazoles (**19a-c**)

2.4 Biological activity of 1,3,4-oxadiazole derivatives

The recent emergence of drug resistance when treating infectious diseases has underlined the need for new, safer, and more efficient antimicrobial agents. Many researchers have reported excellent antimicrobial activity for compounds containing the 1,3,4-oxadiazole core. Recently, Oliveira and co-workers reported synthesis and antistaphylococcal activity of 1,3,4-oxadiazolines (**20)** against strains of *Staphylococcus aureus*, resistant to methicillin and amino glycosides (MARSA), and that encode efflux proteins (multidrug drugs resistant—MDR). The compounds (**20)** showed efficient antistaphylococcal activity at 4 to 32 μg/mL, making all the compounds 2–8 times more active than the standard drug chloramphenicol Figure 1. The compound 2-(2-naphthyloxymethyl)-5-phenoxymethyl-1,3,4-oxadiazole (**21**) exhibits anti-mycobacterial activity at a minimum inhibitory concentration of 6.25 μg/mL (Figure 1). Anti-mycobacterial activity against *Mycobacterium tuberculosis* H37RV was also studied by Kumar and co-workers [40] for a series of di-substituted oxadiazoles (**22)** containing the thiazole unit. The derivative containing the Cl group exhibited excellent results at a MIC of 4 μg/mL (Figure 1).

Yoshida and co-workers described the synthesis and optimization of anti-*Helicobacter pylori* activity for a new series of cephem derivatives. Compound (**23)** exhibited anti *Helicobacter pylori* (13001 and FP1757) activity at a minimum inhibitory concentration of 0.1 μg/mL. Bakal and Gattani investigated anti-tubercular activity for a series of 2,5-disubstituted oxadiazoles against *M. tuberculosis* H337Rv. Compound (**24**) with a MIC50 = 0.04 ± 0.01 μM was comparable with Isoniazid. Compound (**25**) was 7.3-fold more active against *Mycobacterium tuberculosis* H37Rv, and 10.3-fold more active against INH resistant *Mycobacterium tuberculosis* than Isoniazid (Figure 1).

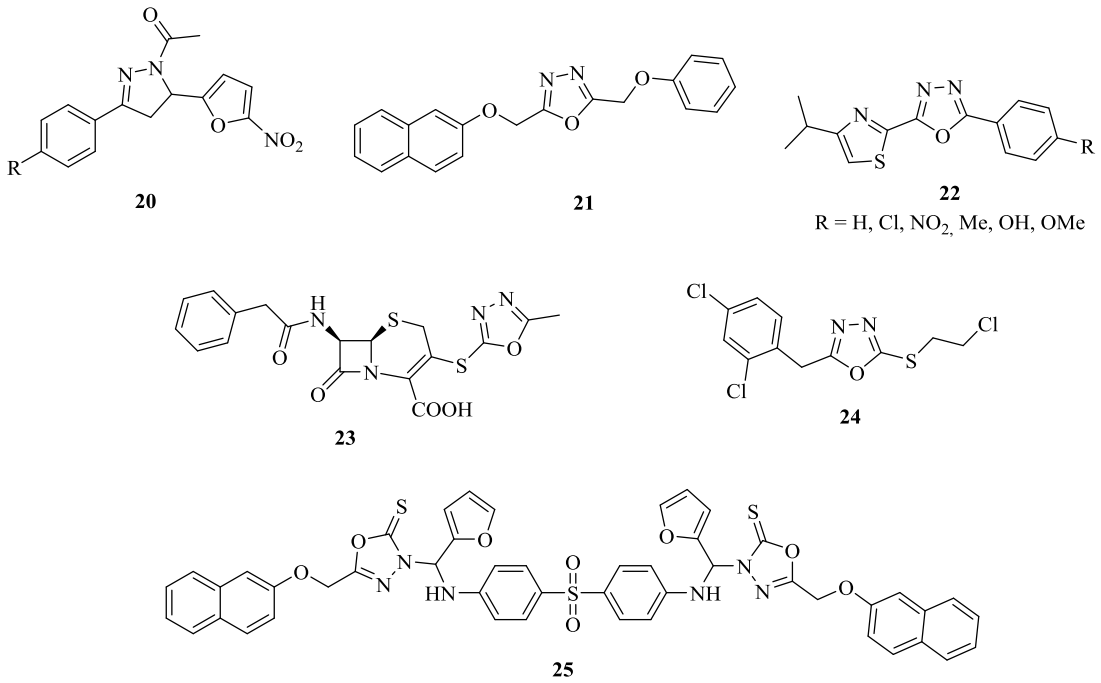


Figure 1: 1,3,4-oxadiazoles with anti-mycobacterial activity

CHAPTER 3

EXPERIMENTAL AND METHODS

3.1 Chemicals and reagents

All the chemicals used were of analytical grade and commercially available from Merck, Sigma–Aldrich, QRëC, Riedel-de Haen, ACROS Organics, Friendemann Schmidt and Bendosen companies. Normal solvents and deuterated solvents were used as received without further purifications.

3.1.1 Chemicals and reagents for synthesis work and identification

Ethyl acetate (EtOAc), *n*-hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), ethanol 99.7% (C_2H_5OH), diethylether ($C_2H_5OC_2H_5$), chloroform ($CHCl_3$) and acetonitrile (C_2H_3N) were produced by AR grade (QRëC, Malaysia); hydrochloric acid (HCl), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), calcium chloride and sodium hydroxide (QrëC, Malaysia); sulfuric acid (Fisher chemicals); carbon disulphide (Riedel-de Haen, Germany); hydrazine monohydrate 65%, tetrabutylammonium bromide (TBAB), benzoic acid, indole-2-carboxylic acid, 4-cholobenzoic acid, 3-nitrobenzoic acid, 2-amino-3-chlorobenzoic acid, *trans*-cinnamic acid, 5-bromo-2-hydroxylbenzoic acid, 4-fluoro-3-nitrobenzoic acid, 3-nitrobenzoic acid, 2-amino-3-chlorobenzoic acid, benzyl chloride and 4-nitrobenzyl chloride (Sigma-aldrich, USA); 3-(4-nitrophenyl)propanoic acid, α-methylcinnamic acid, sodium carbonate, potassium hydroxide and TLC silica gel 60 F254, aluminium sheet, 20cmx 20cm (Merck, Germany); sodium hydrogen carbonate (Bendosen);

sodium sulfate (Friendemann Schmidt, Australia); potassium carbonate, dimethyl sulfoxide-d_6, chloroform-d, acetone-d (Acros, Belgium).

3.2 General experimental methods and Instrumentals

3.2.1 Melting Point Determination

A Stuart Scientific SMP-1 (UK) melting point apparatus at the School of Chemical Sciences, USM was used for melting point measurements of the synthesized compounds.

3.2.2 Thin layer chromatography (TLC)

The progress of all reactions was monitored by TLC. The product and starting materials were spotted on the TLC plates. Two different solvent systems were developed, using chloroform: methanol and hexane: ethyl acetate. The plates were visualised under UV lamp (254 and 365 nm).

3.2.3 Infrared (IR) Spectroscopy

The IR spectra of the compounds were obtained using a FTIR-ATR spectrometer Frontier (Perkin Elmer) at the School of Chemical Sciences, USM. The synthesized compounds were applied directly on the machine, in the frequency range of 4000-650 cm^{-1}.

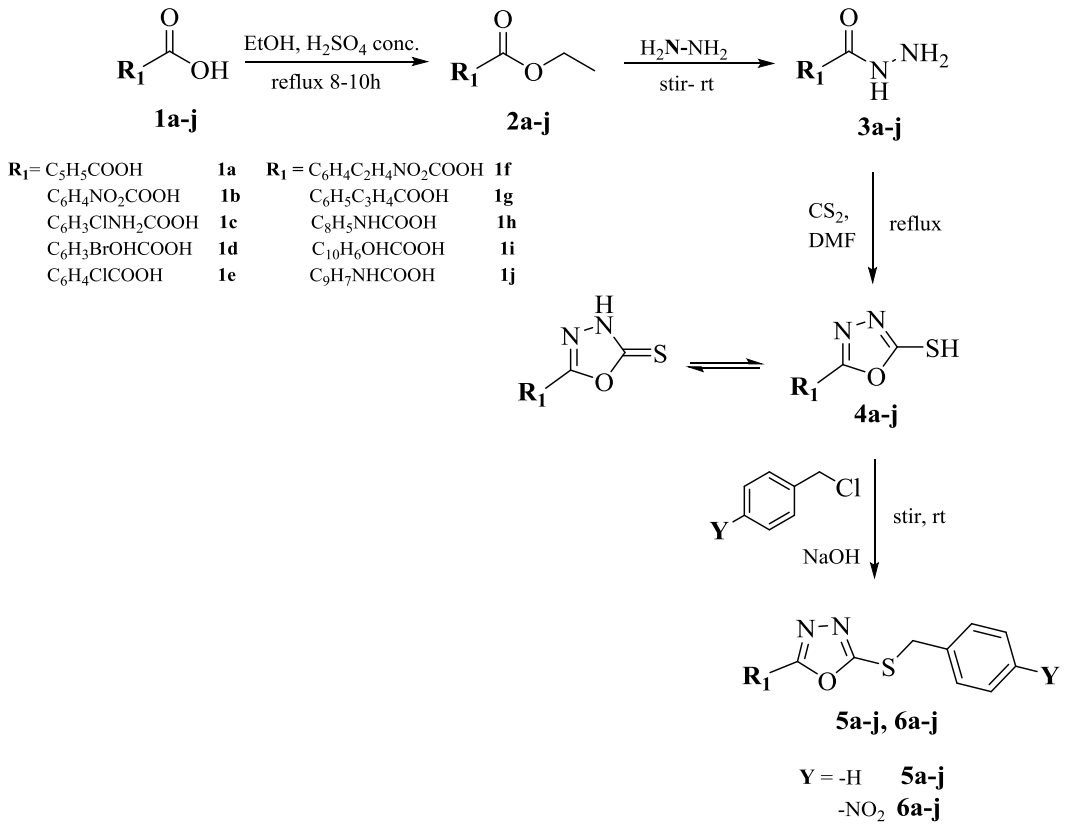
3.2.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra data of all samples were recorded on a 500 FT-NMR Bruker Avance spectrometer at the School of Chemical Sciences, USM. ^{1}H-NMR and 2D NMR experiments (^{1}H-^{1}H COSY, HMBC, HSQC) were recorded on a 500 MHz. ^{13}C

NMR and DEPT experiments (DEPT 90 and DEPT 135) were recorded on a 125 MHz. The chemical shifts (δ, ppm) were calculated with reference to the TMS signal at 0.00 ppm and the signal of deuterated solvents. The synthesized compounds were dissolved in a suitable deuterated solvent and placed in 5 mm x 180 mm^2 NMR glass tubes.

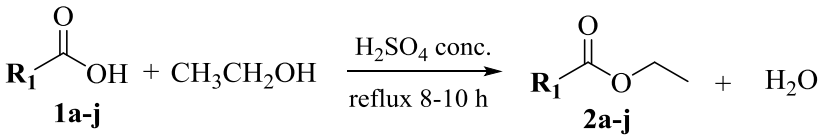
3.3 Synthesis Methods

The overall synthesis of 5-substituted-1,3,4-oxadiazole-2-thiols, (**5a-j**) and (**6a-j**) were carried out according to the previously reported four-step approach as depicted in Scheme 3.1. The ethyl esters (**2a-j**) were either prepared by Fischer esterification of the corresponding carboxylic acids and ethanol (**1a-j**). The reaction of ethyl esters (**2a-j)** with hydrazine monohydrate in ethanol led to the formation of the corresponding acyl hydrazides (**3a-j)**, which were further reacted with carbon disulfide in dimethylformamide under reflux to produce 5-substituted-1,3,4-oxadiazole-2-thiols (**4a-j)**. The alkylation of oxadiazoles (**4a-j)** in the presence of tetrabutylammonium bromide (TBAB) as a phase-transfer catalyst resulted in the formation of the target 2-alkylbenzylsulfanyl-5-substituted-1,3,4-oxadiazole-2-thiols (**5a-j** and **6a-j)**.



Scheme 3.1: Synthesis pathways of 5-substituted-1,3,4-oxadiazole-2-thiol, **5a-j** and **6a-j**.

3.3.1 General procedure for the synthesis of acyl ethyl esters (2a-j).

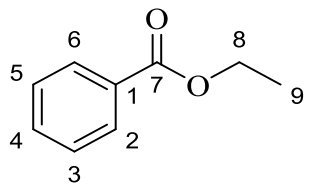


R_1= C_5H_5COOH	**1a**	R_1 = $C_6H_4C_2H_4NO_2COOH$	**1f**
$C_6H_4NO_2COOH$	**1b**	$C_6H_5C_3H_4COOH$	**1g**
$C_6H_3ClNH_2COOH$	**1c**	$C_8H_5NHCOOH$	**1h**
$C_6H_3BrOHCOOH$	**1d**	$C_{10}H_6OHCOOH$	**1i**
$C_6H_4ClCOOH$	**1e**	$C_9H_7NHCOOH$	**1j**

The corresponding carboxcylic acid (**1a-j**) (2g) was refluxed in ethanol (10 mL) and concentrated H_2SO_4 (1.5 mL) for 8-10 hours. After completion of reaction (as evident from TLC profiles), the solvent was evaporated under reduced pressure. The mixture was extracted with ethyl acetate (5 mL x 3). The organic layer was dried

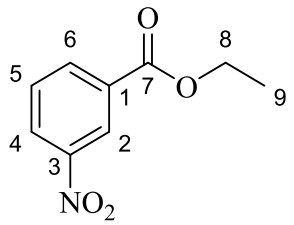
over Na_2SO_4 and concentrated under reduced pressure to afford compounds (**2a-j)** in 80-99% yields without purification.

3.3.1(a) Ethyl benzoate (2a)



White powder, yield: 90 %; mp 133-135 ºC (Lit, 101-102 ºC, Gazizov *et al.*, 2004); IR (υ, cm^{-1}): 2984(Csp^3 -H strech), 1713(C=O), 1440(C=C stretch), 1269(C-O ester); ^1H NMR (500 MHz, DMSO-d_6) δ ppm: 1.38 (t, *J* = 7.1 Hz, 3H, CH_3, H9), 4.37(q, *J* = 7.1 Hz, 2H, -OCH_2, H8), 7.52(t, *J* = 7.6 Hz, 2H, Ar-H3, H5), 7.64(m, 1H, Ar-H4), 8.04(dd, *J* = 1.3, 7.5 Hz, 2H, Ar-H2, H6). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 13.69(C9), 60.54(C8), 128.45(C3, C5), 129.21(C2, C6), 130.64(C1), 132.86(C4), 165.83(C7/C=O).

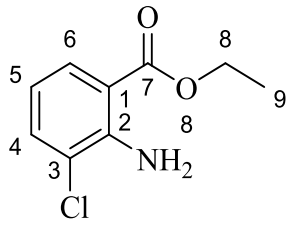
3.3.1(b) Ethyl-3-nitrobenzoate (2b)



Light purple powder, 92 %; mp 145-147 ºC (Lit. 150-152 ºC, Williams *et al.*, 1976); IR (υ, cm^{-1}): 3088(Csp^2-H), 2986(Csp^3-H), 1721(C=O), 1531(N-O), 1258.58(C-O, ester); ^1H NMR (500 MHz, DMSO-d6) δ ppm: 1.36(t, *J* = 7.1 Hz, 3H, -CH_3, H9), 4.39(q, *J* = 7.1 Hz, 2H, -OCH_2, H8), 7.84(t, *J*= 8.0 Hz, 1H, Ar-H5), 8.36(d, *J* = 7.7 Hz, 1H, Ar-H6), 8.49(d, *J* = 5.0 Hz, 1H, Ar-H4), 8.61(s, 1H, Ar-H4). ^{13}C NMR (125 MHz,

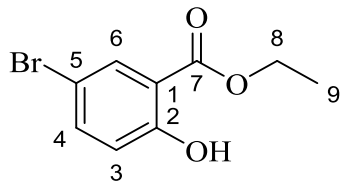
DMSO-d_6) δ ppm: 14.51(C9), 62.14(C8), 123.95(C2), 128.15(C4), 131.18(C5), 131.89(C1), 135.65(C6), 148.84(C3), 164.63(C7, C=O).

3.3.1(c) Ethyl 2-amino-3-chlorobenzoate (2c)



Brown crystal, 71 % ; mp 44-46 ºC (Lit. 98-100 ºC, Keyser *et al.*, 1976; IR (υ, cm^{-1}): 3404(Csp^2-H), 1700(C=O), 1362(C-N), 1224(C-O, ester); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.28(t, *J* = 7.1 Hz, 3H, CH_3, H9), 4.24(q, *J* = 7.1 Hz, 2H, -OCH_2, H8), 6.53(t, *J* = 2.3 Hz, 1H, Ar-H5), 6.84(d, *J* = 2.1 Hz, 1H, Ar-H4), 7.69(d, *J* = 8.6 Hz, 1H, Ar-H6). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 14.61(C9), 60.61(C8), 108.46(C1), 115.29(C4), 115.79(C6), 133.02(C5), 138.99(C3), 152.56(C2), 167.17(C7/C=O).

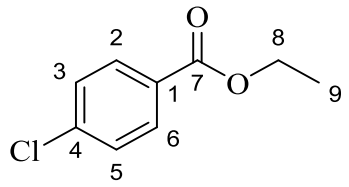
3.3.1(d) Ethyl 5-bromo-2-hydroxybenzoate (2d)



White powder, 96%; mp 144-146 ºC (Lit. 130-135 ºC, Amalendu *et al*., 1981); IR (υ, cm^{-1}): 3004(Csp^2-H), 2584(Csp^3-H), 1700(C=O), 1196(C-O), 1360(O-H, phenol); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.34(t, *J* = 7.1 Hz, 3H, CH_3, H9), 4.33(q, *J* = 7.1 Hz, 3H, OCH_2, H8), 6.80(d, *J* = 8.9 Hz, 1H, Ar-H3), 7.43(dd, *J* = 2.4, 8.9 Hz, 1H, Ar-H4), 7.86(d, *J* = 2.3 Hz, 1H, Ar-H6). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 8.95(C9),

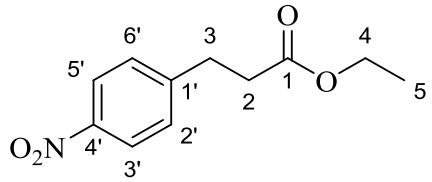
56.75(C8), 105.57(C5), 108.89(C1), 114.34(C3), 126.98(C6), 133.11(C4), 155.40(C2), 163.93(C=O).

3.3.1(e) Ethyl 4-chlorobenzoate (2e)



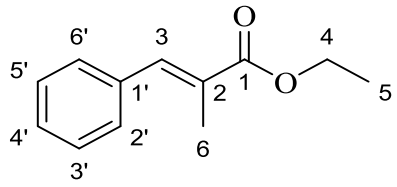
White powder, 57 %; mp 71-73 °C (Lit. 95-97 °C, Amalendu *et al*., 1980); IR (υ, cm^{-1}): 3004(Csp^2-H), 2928(Csp^3-H), 1659(C=O), 1180(C-O, ester), 865(C-Cl), 657(C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.32(t, *J* = 7.1 Hz, 3H, -CH_3, H9), 4.31(q, *J* = 7.1 Hz, 2H, -OCH_2, H8), 7.56(d, *J* = 8.61 Hz, 2H, Ar-H2, H6), 7.94(d, *J* = 8.6 Hz, 2H, Ar-H3, H5). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 14.54(C9), 61.50(C8), 129.20(C3, C5), 130.09(C1), 131.60(C2, C6), 138.27(C4), 166.94(C7).

3.3.1(f) Ethyl 3-(4'-nitrophenyl)propanoate (2f)



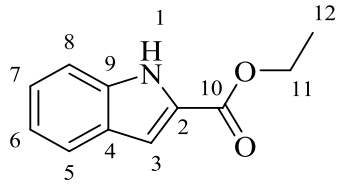
Light yellow powder, 88 %; mp 123-125 °C (Lit. 125-126 °C, Novokreshchennykh *et al*., 1978); IR (υ,cm^{-1}): 3061(Csp^2-H), 1606(C=O), 1522(N-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.35(t, *J* = 7.2, Hz, 3H, CH_3, H5), 2.39(t, *J* = 7.6 Hz, 2H, -CH_2, H2), 2.96(t, *J* = 7.6 Hz, 2H, -CH_2, H3), 4.35(q, *J* = 7.1 Hz, 2H, -OCH_2, H4), 7.48(d, *J* = 8.6 Hz, 2H, Ar-H3', H5'), 8.13(d, *J* = 8.8 Hz, 2H, Ar-H2', H6'). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 14.90(C5), 31.16(C3), 34.65(C2), 64.14(C4), 123.83(C3', C5') 130.04(C2', C6/), 146.40(C1'), 150.11(C4'), 170.76(C1).

3.3.1(g) Ethyl (*E*)-2-methyl-3-phenylacrylate (2g)



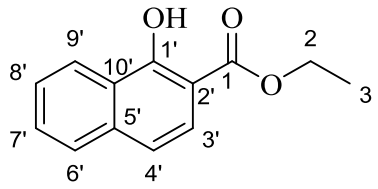
Colourless liquid, 80 %, mp 35 °C (Lit. 33 °C, Petroski *et al*., 2001); IR (υ, cm^{-1}): 3342(Csp^2-H), 2472(Csp^3-H), 2070(Csp^3-H), 1119(C-O, ester); ^{1}H NMR (500 MHz, DMSO-d6) δ ppm: 1.36(t, *J* = 7.1 Hz, 3H, CH_3, H5), 4.31(q, *J* = 7.1 Hz, 2H, -OCH_2, H4) ,2.04(d, *J* = 1.5 Hz, 3H, CH_3-H6), 7.22(s, 1H, CH, H3), 7.37(m, 1H, Ar-H4'), 7.38(t, *J* = 7.3 Hz, 2H, Ar-H3', H5'), 7.49(d, *J* = 7.2 Hz, 2H, Ar-H2', H6'). ^{13}C NMR (125 MHz, DMSO-d6) δ ppm: 14.1(C5), 21.07(C6), 60.70(C4), 128.65(C2', C6'), 129.45(C2), 129.64(C4'), 129.76(C3', C5'), 136.21(C1'), 136.46(C3), 167.1(C1/C=O).

3.3.1(h) Ethyl 1*H*-indole-2-carboxylate (2h)



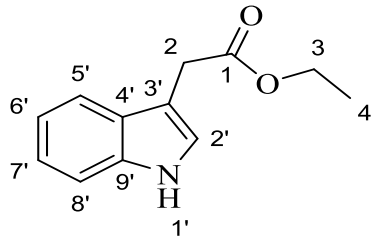
Purple powder, 97%; mp 104-106 ºC (Lit. 118-119 ºC, Fugard *et al*., 2015); IR (υ, cm^{-1}): 3324(Csp^2-H), 2983(Csp^3-H), 1701(C=O), 1241(C-N), 1199(C-O, ester); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.35(t, *J* = 7.2, Hz, 3H, CH_3-H11), 4.35(q, *J* = 7.1 Hz, 2H, -OCH_2-H10), 7.1 (dt, *J* = 1.0, 7.5 Hz, 1H, Ar-H2), 7.15(dd, *J* = 0.8, 1.3 Hz, 1H, Ar-H6), 7.26(dt, *J* = 1.1, 7.1 Hz, 1H, Ar-H7), 7.46(dd, *J* = 0.9, 7.5 Hz, 1H, Ar-H5), 7.66(d, *J* = 8.0 Hz, 1H, Ar-H4), 11.86(s, 1H, -NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 14.77(C12), 60.87(C11), 108.13(C8), 113.03(C3), 120.61(C6), 122.51(C5), 125.07(C7), 127.20(C2), 127.82(C4), 137.83(C9), 161.78(C10/C=O).

3.3.1(i) Ethyl 1'-hydroxy-2'-naphthoate (2i)



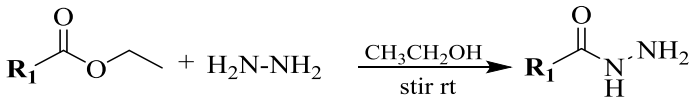
Light purple powder, 91%; mp 68-70 °C (Lit. 46-47 °C, Huang *et al*., 2007); IR (υ, cm^{-1}): 3184(Csp^2-H), 2984(Csp^3-H), 3151(O-H), 1678(C=O), 1214(C-O, ester); ^{1}H NMR (500 MHz, Acetone-d) δ ppm: 1.45(t, *J* = 7.2 Hz, 3H, -CH_3, H3), 4.49(q, *J* = 7.1 Hz, 2H, -OCH_2, H2), 7.31(s, 1H, Ar-H3'), 7.35(t, *J* = 7.5 Hz, 1H, Ar-H7'), 7.53(t, *J* = 7.0 Hz, 1H, Ar-H8'), 7.75(d, *J* = 8.5 Hz, 1H, Ar-H4'), 7.90(d, *J* = 8.5 Hz, 1H, Ar-H6'), 8.54(s, 1H, Ar-H9'), 10.5(s, 1H, -OH, H1'). ^{13}C NMR (125 MHz, Acetone-d) δ ppm: 13.54(C3), 61.82(C2), 111.23(C2'), 114.45(C4'), 123.96(C9'), 126.13(C6'), 127.08(C8'), 129.24(C7'), 132.30(C1'), 137.91(C5'), 156(C10'), 169.78(C1/C=O).

3.3.1(j) Ethyl 2-(1'*H*-indol-3-yl)acetate (2j)



Purple crystal, 82 %; mp 33 °C; IR (υ, cm^{-1}): 3403(Csp^2-H), 2977(Csp^3-H), 1705(C=O), 1361(C-N), 1224(C-O, ester); ^{1}H NMR (500 MHz, $CDCl_3$-d) δ ppm: 1.23(t, *J* = 7.1 Hz, 3H, CH_3, H4), 3.77(s, 2H, CH_2-H2), 4.13(q, *J* = 7.1 Hz, 2H, -OCH_2, H3), 7.1(t, *J* = 8 Hz, 1H, Ar-H8'), 7.13(t, *J* = 7.6 Hz, 1H, Ar-H7'), 7.29(s, 1H, Ar-H2'), 7.41(d, *J* = 8.1, 1H, Ar-H6'), 7.61(d, *J* = 7.9 Hz, 1H, Ar-H5'). ^{13}C NMR (125 MHz, $CDCl_3$-d) δ ppm: 14.17(C5), 31.42(C2), 60.78(C4), 108.64(C3), 111.16(C9'), 118.92(C6'), 119.63(C7'), 122.19(C8'), 123.01(C2'), 128.57(C5'), 136.13(C3), 172.44(C1/C=O).

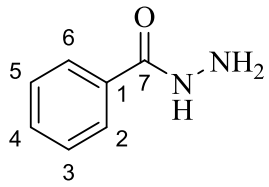
3.3.2 General procedure for the synthesis of acid hydrazide derivatives (3a-j).



2a-j **3a-j**

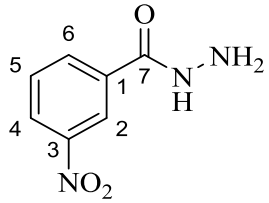
Hydrazine monohydrate (5mmol) was added dropwise to a solution of the corresponding ethyl ester (**2a-j**) (1 mmol) in EtOH (5 mL). The reaction mixture was stirred in room temperature for 12−48 h until completion, as determined by TLC profiles. After completion of reaction, the solvent was evaporated under reduced pressure. The acid hydrazide derivatives (**3a-j**) 42-99% obtained were kept for further reactions without purification.

3.3.2(a) Benzohydrazide (3a)



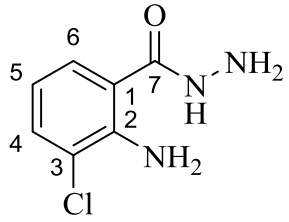
White powder, 98 %; mp 115-117 °C (Lit 116-117 °C, Fahmy *et. al*, 2012); IR (υ, cm^{-1}): 3531(N-H), 1659(C=O), 1385(C-N); ^{1}H NMR (500 MHz, Acetone-d) δ ppm: 7.46(t, 2H, *J* = 7.6 Hz, ArH3, H5), 7.53(t, 1H, *J* = 7.6 Hz, Ar-H4), 7.89(d, 2H, *J* = 7.4 Hz, Ar-H2, H6), 9.92(s, 1H, -NH). ^{13}C NMR (125 MHz, Acetone-d) δ ppm: 127.52(C3, C5), 128.39 (C2, C6), 131.27(C1), 134.30(C4), 159.16(C7/C=O).

3.3.2(b) 3-nitrobenzohydrazide (3b)



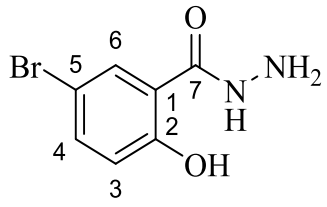
Brown powder, 99 %; mp 100-102 °C; (Lit. 105-108 °C, Kumar *et al*., 2008); IR (υ, cm^{-1}): 3503(N-H), 2930(Csp^2-H), 1655(C=O), 1501(N-O), 1255(C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.77(t, *J*= 8.0 Hz, 1H, Ar-H5), 8.27(dd, *J* = 1.13, 7.7 Hz, 1H, Ar-H6), 8.37(dd, 1H, *J* = 1.4, 8.3 Hz, Ar-H4), 8.64(t, *J* = 1.75 Hz, 1H, Ar-H2). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 123.95(C2), 128.15(C4), 131.18(C5), 131.89(C1), 135.65(C6), 148.84(C3), 164.63(C7/C=O).

3.3.2(c) Synthesis of 2-amino-3-chlorobenzohydrazide (3c)



Light brown crystal, 71 %; mp 46-48 °C; IR (υ, cm^{-1}): 3404(N-H), 2992(Csp^2-H), 1700(C=O), 1362(C-N), 865(C-Cl); ^{1}H NMR (500 MHz, Acetone-d) δ ppm: 6.57(dd, *J* = 2.1, 8.7 Hz, 1H, Ar-H5), 6.86(d, *J* = 2.0 Hz, 1H, Ar-H4), 7.80 (d, *J* = 8.7 Hz, 1H, Ar-H6). ^{13}C NMR (125 MHz, Acetone-d) δ ppm: 108.46(C1), 115.29(C4), 115.79(C6), 133.02(C5), 138.99(C3), 152.56(C2), 167.17(C7/C=O).

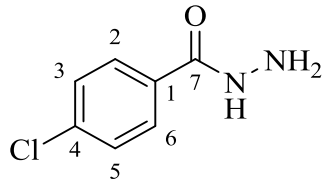
3.3.2(d) Synthesis of 5-bromo-2-hydroxybenzohydrazide (3d)



Light brown powder, 42 %; mp 130-132 °C (Lit. 217-218 °C, Fox *et al*., 1952); IR (υ, cm^{-1}): 3485(N-H), 3327(-NH_2), 3004(Csp^2-H), 1657(C=O), 1385(O-H), 1255(C-N), 658(C-Br); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.80 (d, *J* = 8.9 Hz, 1H, Ar-H3), 7.43 (dd, *J* = 2.4, 6.4 Hz, 1H, Ar-H4), 7.86 (d, *J* = 2.3 Hz, 1H, Ar-H6). ^{13}C NMR (125 MHz,

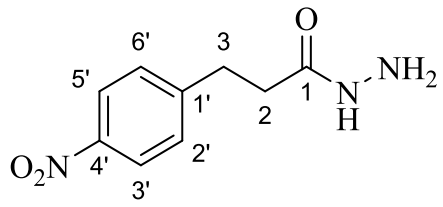
$CDCl_3$) δ ppm: 105.57(C5), 108.89(C1), 114.34(C3), 126.98(C6), 133.11(C4), 155.40(C2), 163.93(C=O).

3.3.2(e) Synthesis of 4-chlorobenzohydrazide (3e)



Cream powder, 98 %; mp 142-144 °C (Lit. 145-148 °C, Kumar *et al*., 2008); IR (υ, cm^{-1}): 3663(O-H), 3408((N-H), 3318(-NH_2), 3004(Csp^2-H), 1659(C=O), 1385(C-N), 1089(C-N, amide), 865(C-Cl stretching), 657(C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.56(d, *J* = 8.6 Hz, 2H, Ar-H2, H6), 7.94(d, 2H, *J* = 8.61 Hz, Ar-H3, H5). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 128.85(C3, C5), 129.33(C2, C6), 132.46(C1), 136.37(C4), 165.33(C7).

3.3.2(f) Synthesis of 3-(4'-nitrophenyl)propanehydrazide (3f)



Light brown powder, 89 %; mp 120-122 °C; IR (υ, cm^{-1}): 3488(N-H), 2929(Csp^2-H), 1655(C=O), 1590(C=C), 1503(N-O), 1438(C-H), 1385(C-H), 657(C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.39(t, *J* = 7.6 Hz, 2H, -CH_2, H2), 2.96(t, *J* = 7.6 Hz, 2H, -CH_2, H3), 7.48(d, *J* = 8.6 Hz, 2H, Ar-H3', H5'), 8.13(d, *J* = 8.8 Hz, 2H, Ar-H2', H6'). ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 31.16(C2), 34.65(C3), 123.83(C3', C5') 130.04(C2', C6'), 146.40(C1'), 150.11(C4'), 170.76(C1).