# SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLES BEARING AN INDOLE RING

By

**KONG KIAN LIANG**

A project report submitted to the Department of Chemical Science,

Faculty of Science,

Universiti Tunku Abdul Rahman,

In partial fulfilment of the requirement for the degree of

Bachelor of Science (Hons) Chemistry

April 2019

## ABSTRACT

### SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLE BEARING AN INDOLE RING

**Kong Kian Liang**

1,3,4-oxadiazoles have been widely studied by many researchers in various fields. Due to its heterocyclic structure, 1,3,4-oxadiazoles possess of many biological and pharmacological activities such as antibacterial, anticancer, anti-inflammatory, antimalarial and antioxidant activities. In this project, carboxylic acid hydrazide was synthesized and act as a starting material for the synthesis of 1,3,4-oxadiazoles. A total of four 1,3,4-oxadiazoles have been synthesized. The structure of carboxylic acid hydrazide and 1,3,4-oxadiazoles were elucidated and characterized through various instrumental analysis such as melting point, FT-IR, $^{1}H$ NMR, $^{13}C$ NMR, DEPT, HMQC and HMBC. The determination of antioxidant activity of the carboxylic acid hydrazide and 1,3,4-oxadiazoles was carried out through DPPH assay with BHT as standard reference. All the compounds synthesized exhibit weak antioxidant activity (> 200 ppm) as compared to BHT.

## ABSTRAK

**SINTESIS, PENCIRIAN DAN AKTIVITI ANTIOKSIDAN 1,3,4-OXADIAZOLE DENGAN INDOLE RING**

**Kong Kian Liang**

Senyawa yang mengandungi 1,3,4-oxadiazole telah digajikan oleh para-para penyelidik dalam bidang-bidang yang penting. 1,3,4-oxadiaozle mempunyai pelbagai aktiviti yang berkaitan dengan biologi kerana 1,3,4-oxadiazole adalah sebuah senyawa heterosiklik. Contoh-contoh aktiviti biologi termasuk antibakteria, antikanser, anti-radang, antimalarial, dan antioksidan. Dalam projek ini, carboxylic acid hydrazide telah disintesis sebagai bahan permulaan dalam sintesis 1,3,4-oxadiazole. Terdapat empat 1,3,4-oxadiazole telah disintesis. Stuktur-struktur bagi senyawa yang disintesis telah dicirikan dengan pelbagai analisis seperti melting point, FT-IR, $^{1}$H NMR, $^{13}$C NMR, DEPT, HMQC dan HMBC. Aktiviti antioksidan carboxylic acid hydrazide dan pelbagai 1,3,4-oxadiazole telah dinilaikan dan dikenalpastikan dengan kaedah DPPH yang menggunakan BHT sebagai standard. Semua senyawa yang disintesis mempamerkan aktiviti antioksidan yang lemah (> 200 ppm) berbanding dengan BHT.

## ACKNOWLEDGEMENT

First and foremost, I would like to thank my supervisor, Dr. Sim Kooi Mow, for his patience, guidance and support thoughout the period of my final year project,. His kindness and willingness to share his knowledge and giving advices has helped me to gain more knowledge that beyond our lectures and syllabus.

Besides, I would like to thank Mr. Leong Thung Lim as well as other lab assistants and lab officers for sharing their knowledge and lending a helping hand throughout my final year project.

Next, I would like to thank my friends, Jien Lee and Jun Ni for showing their kindness, patience and support through the period of my final year project. Lastly, I would like to thank my family and friends for the encouragement and their support for my final year project.

## DECLARATION

I hereby declare that the project report is based on the original work except for quotations, and citation which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name:

Date:

## APPROVAL SHEET

This thesis report entitled **"SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLE BEARING AN INDOLE RING"** was prepared by **KONG KIAN LIANG** and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Chemistry at Universiti Tunku Abdul Rahman.

Approved by:

_____ Date: _____

(DR. SIM KOOI MOW)

Supervisor

Department of Chemical Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

**PERMISSION SHEET**

It is hereby certified that **KONG KIAN LIANG** (ID No. 16ADB05307) has completed this final year project entitled **"SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLE BEARING AN INDOLE RING"** supervised by Dr. Sim Kooi Mow from Department of Chemical Science, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscripts of these research findings for publishing in any form, if I do not prepare it within six (6) month from this date, provided that my name is included as one of the authors for this articles. The arrangement of the name depends on my supervisor.

Yours truly,

_____

(KONG KIAN LIANG)

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS / ABBREVIATION

| | |
|---|---|
| $gmol^{-1}$ | Molecular weight |
| mmol | Millimol |
| nm | Wavelength |
| δ | Chemical shift |
| ppm | Part per million |
| Hz | Hertz |
| J | Coupling constant |
| s | Singlet |
| d | Doublet |
| dd | Doublet of doublet |
| $IC_{50}$ | Effective concentration for 50% reduction of activity |
| $R_f$ | Retention factor |
| NMR | Nuclear Magnetic Resonance |
| DEPT | Distortionless Enhancement by Polarization Transfer |
| HMQC | Heteronuclear Multiple Quantum Correlation |
| HMBC | Heteronuclear Multiple Bond Correlation |
| FT-IR | Fourier Transform Infrared Spectroscopy |
| TLC | Thin Layer Chromatography |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl hydrate |

# CHAPTER 1

# INTRODUCTION

## 1.1 Indole

Indole, also known as benzopyrrole, with the chemical formula of $C_8H_7N$ is a heterocyclic compound with a bicyclic structure. The general structure of an indole consists of a pyrrole ring together with an aromatic benzene ring. It is a white colour solid in appearance with the molecular weight of 117.15 g $mol^{-1}$. Indole containing compounds can be found naturally within various plant sources or synthesize synthetically from laboratories through different methods. Indole compounds commonly exhibit strong biological and pharmacological activities. For example like antibacterial, anticancer, anti-inflammatory, antifungal and more. Hence, indole compounds have various applications in agrochemicals, pharmaceuticals, and material science. Figure 1.1 shows the basic structure of an indole. (Siddalingamurthy, 2014)

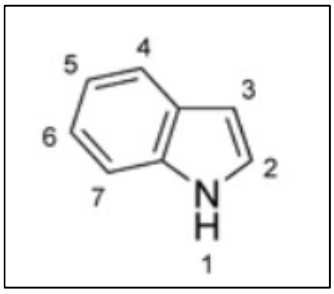


**Figure 1.1: Structure of an indole**

## 1.2 Hydrazide

In organic chemistry, hydrazide is a class of organic compound containing an active functional group with the general structure of R–C(=O)$NHNH_2$. This structure is composed of a covalent bond between two nitrogen with four substituents, while at least one of the substituent is an acyl group. Hydrazides can be synthesized by the reaction between hydrazine and different acyl derivatives like acyl halides, esters, and cyclic anhydrides.

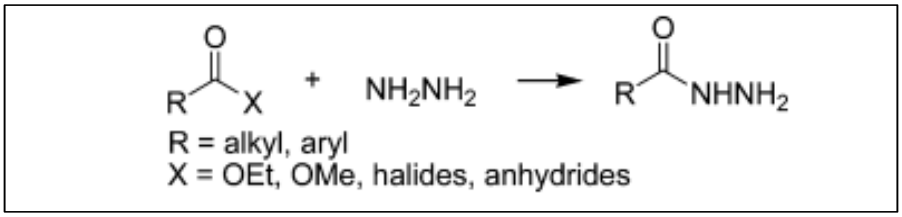


**Figure 1.2: General equation for the synthesis of hydrazide.**

As hydrazides are a reactive substance, they can be used as a good bidentate ligand and form a transition metal complexes either in amide or imide form, depending on the acidity of the medium. There are a wide range of applications for the hydrazides, such as manufacturing medicines, polymers, and plant chemical preservers. Besides, hydrazides and their derivatives are also an important part of the molecules in various heterocyclic rings, as they show some useful applications in antibacterial agents, dyes and pharmaceutical area. Apart from that, hydrazides analogues made by cycloaddition or cyclization with different reagents also possess some biological activities like anticancer,

anticonvulsant, antidepressant, antimalarial and anti-inflammatory activities. (Majumdar et al., 2014)

### 1.3 Oxadiazole

Oxadiazole is a five-membered ring heterocycle compounds in azole family with the molecular formula of $C_2H_2N_2O$. Oxadiazole having a molecular weight of 70.051 g $mol^{-1}$ and their basic structure consist of two double bonds, two nitrogen atoms, and a single oxygen atom. Depends on the position of the nitrogen atom in the oxadiazole ring, it can exist in four different isomeric forms. Among these isomers, 1,2,4-oxadiazole and 1,3,4-oxadiazole were widely investigated due to their chemical and biological significances. Figure 1.3 shows the different isomeric forms of oxadiazole. (Kaur and Kaur, 2018)

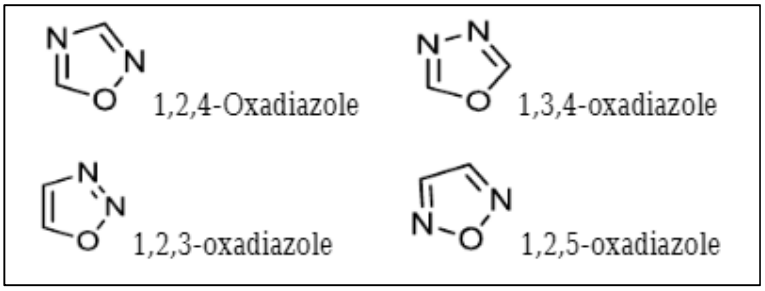


**Figure 1.3: Structure of oxadiazole isomers**

### 1.3.1 1,3,4-oxadiazole

1,3,4-Oxadiazole is one of the isomers of oxadiazole, a five-membered heterocyclic ring consisting of one oxygen atom and two nitrogen atoms. The structure of the 1,3,4-oxadiazole closely resembles to the structure of furan, whereby two methane groups (-CH=) in the furan were replaced by two pyridine type of nitrogen atoms (-N=). In the year 1965, Ainsworth was the one who firstly prepared 1,3,4-oxadiazole by the thermolysis reaction of ethylformate (formally known as hydrazine) at atmospheric pressure, the physical state of the 1,3,4-oxadiazole synthesized is liquid in nature. (Patel et al., 2014). The conventional pathway for the synthesis of 1,3,4-oxadiazoles involves the intermolecular condensation reaction between acid hydrazides or hydrazides derivatives with the carboxylic acid or acid chlorides. Figure 1.2.1 shows the basic structure of 1,3,4-oxadiazole. (Bala et al., 2014)


**Figure 1.4: Basic structure of 1,3,4-oxadiazole**

### 1.3.2 Biological activities of 1,3,4-oxadiazole

1,3,4-Oxadiazole is a prominent compound in oxadiazole family, mainly due to their heterocyclic structure that possesses significant biological and pharmacological activities. Hence, 1,3,4-oxadiazole is commonly used in the development of new pharmaceutical drugs as they exhibit some biological activities like antibacterial, anticancer, anticonvulsant, anti-inflammatory, antimalarial, and analgesic properties. Some of the biological activities of 1,3,4-oxadiazole are discussed below. (Patel et al., 2014)

#### 1.3.2.1 Antibacterial activity

A series of 1,3,4-oxadiazole compounds,1-(4-methoxy-phenyl)-3-[5-(substituted phenyl)-1,3,4-oxadiazol-2-yl]propan-1-one were synthesized and biological evaluated for their antibacterial activities. Some microorganisms like Gram-positive bacteria (*Staphylococcus epidermis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were used in the evaluation of antibacterial properties. All of the compounds synthesized found to have a positive result against *Pseudomonas aeruginosa* and *Staphylococcus aereus*, which indicate these compounds have strong antibacterial activity. (Bala et al., 2014)

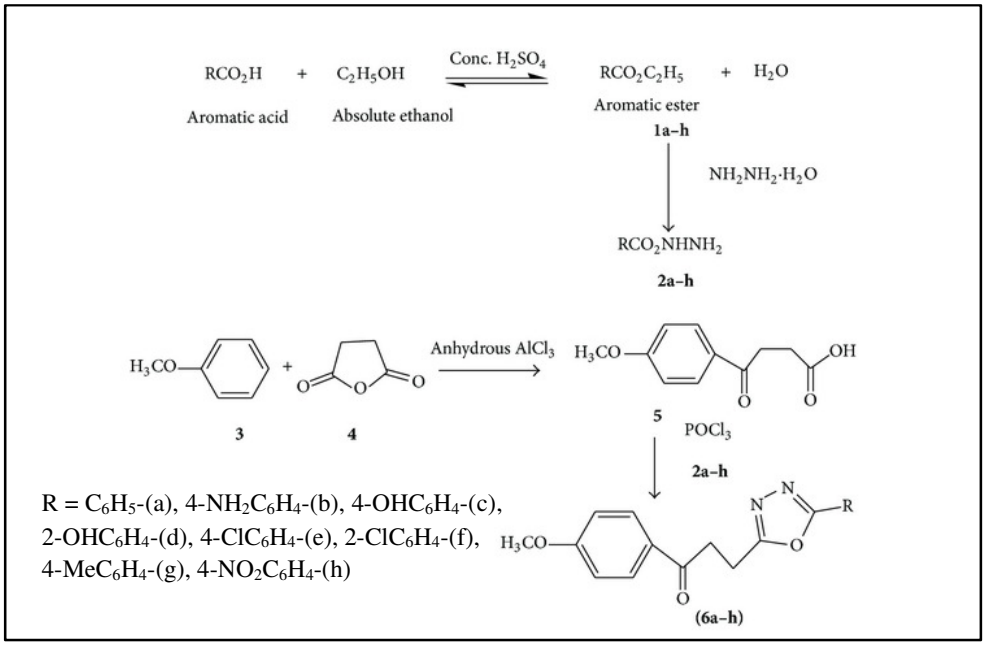


**Figure 1.5: Synthesis of 1,3,4-oxadiazoles by Bala et al.**

### 1.3.2.2 Anti-inflammatory activity

The synthesis of a series *N*-((5-substituted-1,3,4-oxadiazol-2-yl)methyl) benzo[*d*]thiazol-2-amine were screened for their anti-inflammatory activities. Carrageenan-induced paw edema method was used for the evaluation of the anti-inflammatory activity. The result shows that the 1,3,4-oxadiazole containing compounds with aryl-substitution at position C-2 possess higher edema inhibition compared to the one with aliphatic or thiol substitutions at the same position. But overall all of the 1,3,4-oxadiazoles synthesized showed significant anti-inflammatory activities. (Iyer et al., 2016)

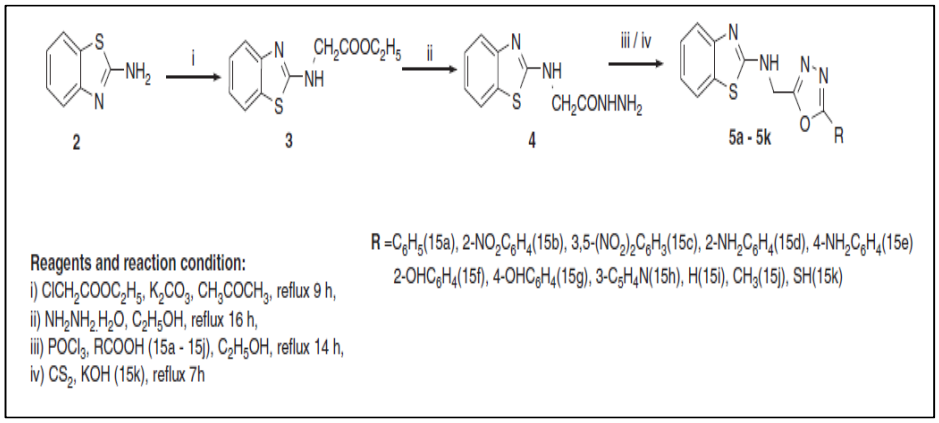


**Figure 1.6: Synthesis of 1,3,4-oxadiazoles by Iyer et al.**

### 1.3.2.3 Anticancer activity

A series of 5-(pyridine-4-yl)-*N*-substituted-1,3,4-oxadiazol-2-amines were synthesized and evaluated for their in vitro anticancer activity. These compounds were prepared by using isonicotinic acid and hydrazides, the resulted hydrazinecarbothioamide derivatives undergoes cyclization and give rise to 1,3,4-oxadiazole derivatives. This series of compounds were tested against a normal fibroblast cell and six human cell cancer lines, such as human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2) and more. As the result, all the compounds exhibit significant anticancer activity against these human cancer cell lines. (Megally Abdo and Kamel, 2015)

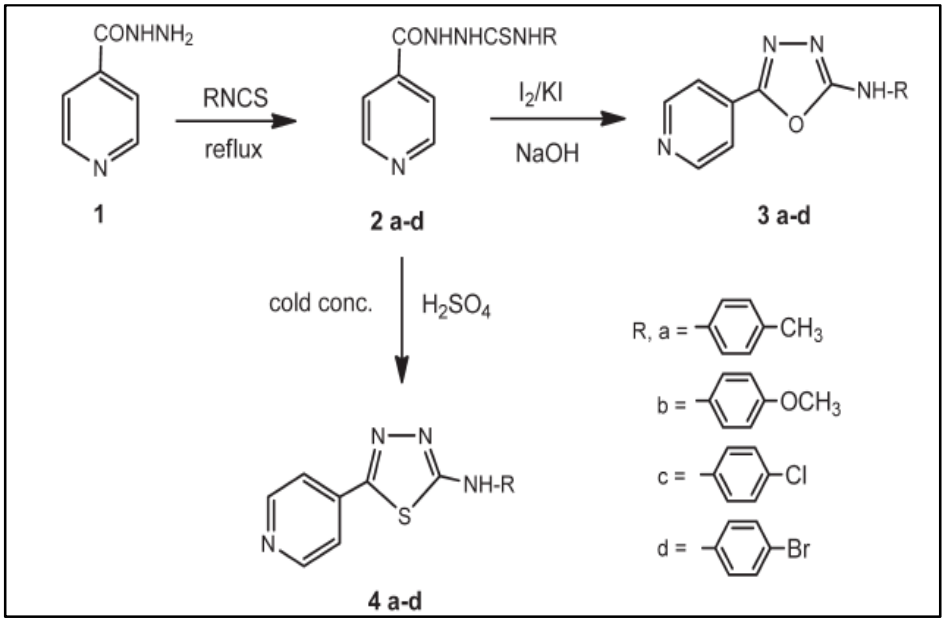


**Figure 1.7: Synthesis of 1,3,4-oxadiazole derivatives by Megally Abdo and Kamel**

### 1.4 Antioxidant activity

The antioxidant is an important compound that inhibits the oxidation process of the substrate by reducing or neutralize free radicals, so that body cells can be protected against radical-induced oxidative injury. Free radicals are either atoms, ions, or molecules together with unpaired electrons, which can be found inside the human body. These radicals with high reactivity are able to cause DNA bases modification, peroxidation of lipid molecules, protein damages, and ultimately body cells and tissues damages. Several diseases like cardiovascular diseases, cancers, diabetes were found to be related to high levels of free radicals. (Barbuceanu et al., 2014). Hence, antioxidants play an important role in the functions of body defence systems against various diseases.

Antioxidants can be either occurs naturally or synthesized in laboratories. Natural antioxidants can be found from different spices or herbs, as well as fruits and vegetables. The common antioxidants insides these sources are vitamin C (ascorbic acid), vitamin E (tocopherol) and polyphenolic compounds. (Augustyniak et al., 2010). While the antioxidants that can be produced synthetically are tert-butylated hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). (Hossain et al., 2008)

There are numerous methods available for the determination of antioxidant activity for the samples of interest. These methods can be categorized into two different types, which are in vivo and in vitro methods. The example of *in vivo* methods are reduced glutathione estimation (GSH), superoxide dismutase method (SOD) and LDL assay. While 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay, nitric oxide scavenging assay and hydrogen peroxide scavenging ($H_2O_2$) assay are some of the methods for the evaluation of in vitro antioxidant activity. (Alam, Bristi and Rafiquzzaman, 2013)

### 1.4.1. DPPH assay

DPPH (2,2,-diphenyl-1-picrylhydrazyl) with the chemical formula of $C_{18}H_{12}N_5O_6$ is a stable free radical used in this *in vitro* antioxidant assay. High stability of DPPH free radical is because of the delocalization of electron over the entire DPPH structure, so that the DPPH molecules is incapable to dimerize. The structure of DPPH was shown in Figure 1.7.

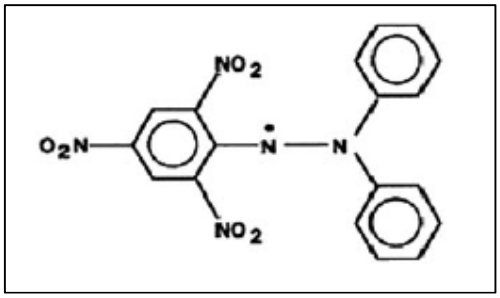


**Figure 1.8: Structure of DPPH**

The principles of DPPH assay is based on the measurement of antioxidants radical scavenging capacity. DPPH molecule will be reduced upon the acceptance of either hydrogen radical or an electron from the antioxidant molecules, and result in the formation of diamagnetic molecules with high stability. The maximum wavelength for DPPH assay is around 520 nm due to the presence of odd electrons from DPPH which shows strong absorption at this wavelength. Once the hydrogen radical pairs off with DPPH free radical, the intensity of the absorption decrease and followed by decolourization. Hence the absorbance will change accordingly based on the amount of antioxidant available. (Kedare and Singh, 2011)

## 1.5 Objectives of the project

1. To synthesize a carboxylic acid hydrazide and a series of 1,3,4-oxadiazoles bearing an indole ring.
2. To characterize the structure of carboxylic acid hydrazide and 1,3,4-oxadiazoles using different spectroscopic methods such as FT-IR, NMR, DEPT, HMQC, and HMBC.
3. To study the antioxidant activity of the carboxylic acid hydrazide and 1,3,4-oxadiazole synthesized by using DPPH assay.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Synthesis of carboxylic acid hydrazide

Various carboxylic acid hydrazides were synthesized via the reaction between the ester (or carboxylic acid) with hydrazine monohydrate ($N_2H_4.H_2O$). Some spectrometric methods like NMR spectrometry and FT-IR spectrometry were used for the characterization of the compound. Carboxylic acid hydrazides can be synthesized by using the conventional method and microwave method.

### 2.1.1 Conventional method

Conventional method for the synthesis of carboxylic acid hydrazide involves nucleophilic addition of hydrazine monohydrate to an ester. Hydrazine will act as a nucleophile and attack the carbonyl group of the ester, leaving an alkoxide anion. The alkoxide anion will deprotonate the intermediate structure, producing carboxylic acid hydrazide.

According to Gadegoni and Manda (2013), a mixture of (1H-indol-3-yl)-acetic acid ethyl ester (0.01 mol) and hydrazine hydrate (0.025 mol) was added with ethanol. The reaction mixture was refluxed for around 7 hours. The resultant mixture was cooled to room temperature, followed by filtration. The crude solid product was recrystallized by using hot ethanol and give rise to a pale yellow solid product, known as (1H-indol-3-yl)-acetic acid hydrazide. The melting

point was measured and the structure of the product was characterized by NMR spectroscopy and FT-IR spectroscopy.

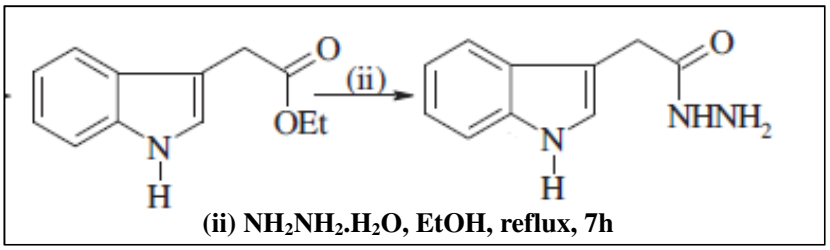


**Figure 2.1: Synthesis of carboxylic acid hydrazide by Gadegoni and Manda**

### 2.1.2 Microwave method

Various types of carboxylic acid hydrazides have been synthesized by treating the mixture of organic carboxylic acids and hydrazine monohydrate under microwave irradiation at 900 Watts (2.45 GHz) for 60-200 seconds, without the usage of any solvent. The resultant reaction mixture was cooled to -20°C, followed by lyophilisation at -50°C. The crude products obtained was recrystallized by using methyl alcohol. The percentage yield of the carboxylic hydrazides synthesized by irradiation of microwave is around 79% to 90%. The characterization of these carboxylic acid hydrazides was done by using NMR and IR spectroscopy. (Saha et al., 2010)

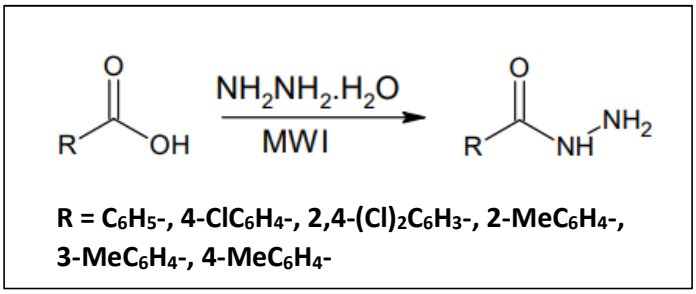


**Figure 2.2: Synthesis of carboxylic acid hydrazide by Saha et al.**

## 2.2 Synthesis of 1,3,4-oxadiazole

1,3,4-Oxadiazole derivatives have been synthesized via a condensation reaction between carboxylic acid hydrazides and various aromatic acids. 1,3,4-oxadiazole synthesized are subjected to characterization by using various spectroscopy methods such as FT-IR spectroscopy, mass spectroscopy, NMR spectroscopy, and other elemental analysis. The application of 1,3,4-oxadiazole has been widely studied by many researchers, especially for their pharmaceutical and biological activities. (Kumar Kolli, 2016) 1,3,4-Oxadiazole can be synthesized by using either the conventional method or microwave method.

### 2.2.1 Conventional method

Based on the research of Jha et al. (2010), a mixture of hydrazides, carbon disulphide, and potassium hydroxide was added with 15 mL of ethanol. The reaction mixture was refluxed for 12 hours until there is no evolution of $H_2S$ gas. The solvents in excess were removed under vacuum and the crude product was dissolved in water, followed by acidification with 10% hydrochloric acid to pH 5. The product was then filtered, dried and recrystallized by using ethanol. The completion of the reaction was monitored by thin layer chromatography (TLC) and IR-spectroscopy. The product namely 5-(substituted)-2-thio-1,3,4-oxadiazoles were characterized by using FT-IR and NMR spectroscopies.

Another series of 1,3,4-oxadiazole derivatives namely 3,5-disubstituted-1,3,4-oxadiazoles were synthesized by treating aromatic acid hydrazides with aromatic acid. The mixture was refluxed for 8 hours after the addition of 5 mL of phosphoryl chloride ($POCl_3$), and the reaction was monitored by using thin layer chromatography (TLC). After the reaction completed, the resultant mixture was poured into the crushed ice and treated with sodium bicarbonate solution to make the mixture basic. The precipitated solid was filtered, dried and recrystallized by using ethanol. The melting point was determined by using melting point apparatus and the characterization of the structure was done by using NMR and FT-IR spectroscopy. (Jha et al., 2010)

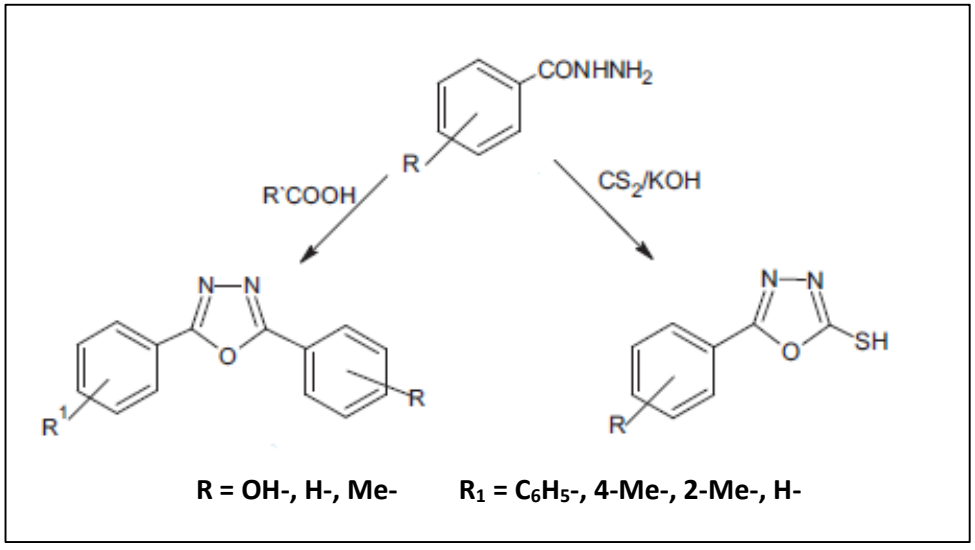


**Figure 2.3: Synthesis of 1,3,4-oxadiazoles by Jha et al.**

According to Mickevičius, Vaickelionienė and Sapijanskaitė (2009), a series of 1,3,4-oxadiazoles namely 1-aryl-4-(1,3,4-oxadizol-2-yl)pyrrolidin-2-ones were synthesis by treating acid hydrazides (10 mmol) with triethyl orthoformate (80 mmol). The reaction mixture was refluxed for 8 hours. After the completion of the reaction, the resultant mixture was cooled and the crystal was filtered off from the mixture. The filtered crystal was then washed with ether and dried in the oven. Thin layer chromatography (TLC) was used to determine the purity of the compounds, while FT-IR and NMR spectroscopies, and mass spectrometry were used to characterize the 1,3,4-oxadiazole derivatives synthesized.

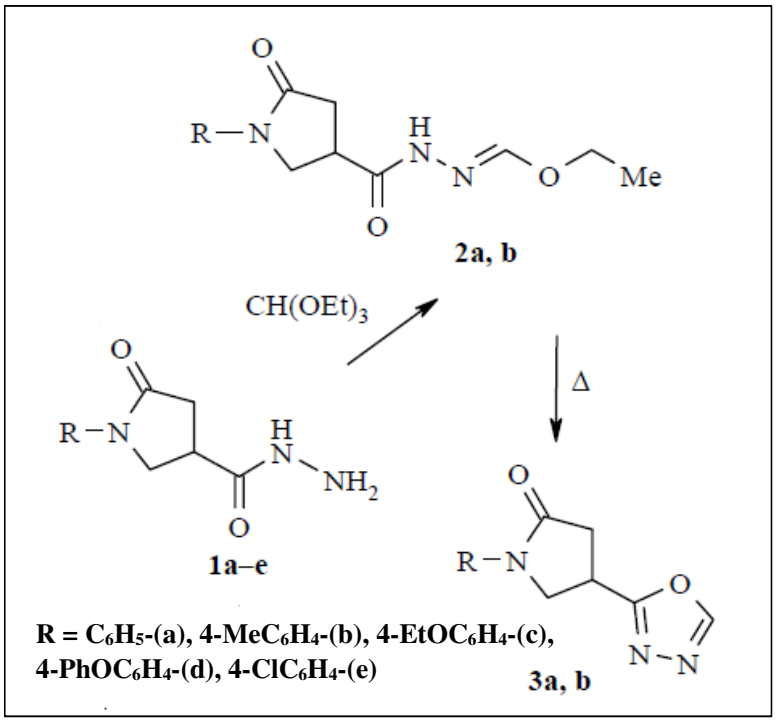


**Figure 2.4: Synthesis of 1,3,4-oxadiazole derivatives by Mickevičius, Vaickelionienė and Sapijanskaitė**

A mixture of benzoic acid (0.008 mol) and thiophene-2-carbohydrazide (0.0078 mol) was heated under reflux at 120°C for around 3-4 hours, after the addition of 7.3 mL phosphorous oxychloride (0.078 mmol). The $POCl_3$ in excess was used as a solvent in the reaction. After the reaction is completed, the residue was poured into ice and neutralized by using sodium bicarbonate ($NaHCO_3$) solution. The crude precipitate was filtered, dried in the oven and recrystallized by using ethanol. The name of the compound synthesized is known as 3,5-disubstituted-1,3,4-oxadiazoles. The obtained 1,3,4-oxadiazole derivatives structure was elucidated by using NMR spectroscopy, FT-IR spectroscopy, and LC-MS. (Kumar Kolli, 2016)

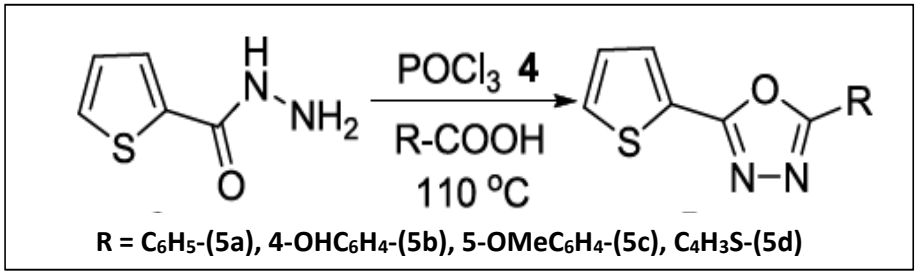


**Figure 2.5: Synthesis of 1,3,4-oxadiazoles by Kumar Kolli**

According to Husain and Ajmal (2009), a novel series of 1,3,4-oxadiazole compounds, namely 2-[3-(4-bromophenyl)propan-3-one]-5-(substituted phenyl)-1,3,4-oxadiazoles) have been synthesized by treating 3-(4-bromobenzoyl)propionic acid with acid anhydrides in phosphorus oxychloride. The reaction mixture was refluxed for 5 hours and cooled to room temperature after the reflux is completed. After the reaction mixture was poured into the ice and neutralized, the resultant precipitate was filtered off under vacuum, washed with distilled water and dried. Methanol was used to recrystallize the solid product. The structure of the product was analysed and characterized by $^{1}H$ NMR, FT-IR and mass spectrometry.

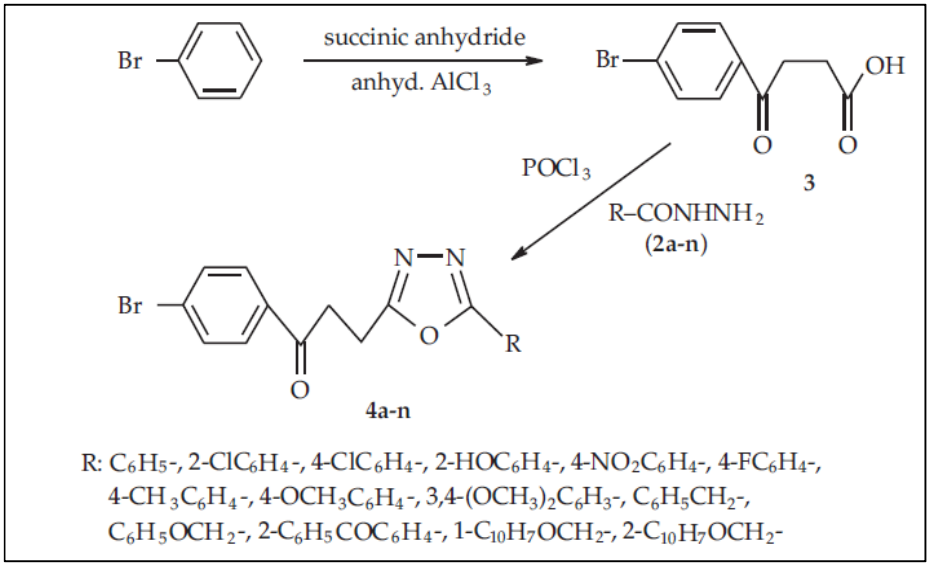


**Figure 2.6: Synthesis of 1,3,4-oxadiazoles by Husain and Ajmal**

### 2.2.2 –Microwave assisted method

According to Modi and Modi (2012), a mixture with equimolar of 5-(4-nitro)phenyl-3H-1,3,4-oxadiazoline-2-thione, trimethylamine and 1-bromo tetradecane added with 1 mL of absolute ethanol was subjected to microwave irradiation for 55 seconds at 760 W. The solvent was removed by using rotary evaporator and the residue was poured into a beaker of water. The precipitate formed was recrystallized from a mixture of ethanol and water in the ratio of 1:1. The compound obtained is named as 5-(4-nitro)phenyl-2-n-tetradecylthio-1,3,4-oxadiazole. The purity of the compounds was determined by thin layer chromatography (TLC) while the structure of the compound was confirmed by using NMR spectroscopy and FT-IR spectroscopy.

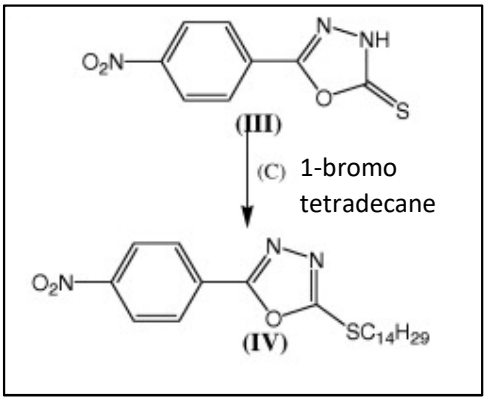


**Figure 2.7: Synthesis of 1,3,4-oxadiazoles by Modi and Modi**

According to the research of Biju et al. (2012), a mixture of isoniazid and aromatic aldehyde, together with 5 drops of DMF (dimethylformamide) was subjected to microwave irradiation for 3 minutes at 300 W. The crude product was filtered, washed with distilled water and recrystallized by using ethanol. Subsequently, the compound synthesized in the previous steps was dissolved in ethanol and added with chloramine-T. The resultant reaction mixture was kept under microwave irradiation for 4 minutes at 300 W. Once again, the solid product was filtered, washed with distilled water and undergoes recrystallization by using methanol. The compounds synthesized was known as 2-aryl-5-(4-pyridyl)-1,3,4-oxadiazole. Thin layer chromatography (TLC) was used to determine the purity of compounds, while FT-IR spectroscopy was used to analyse the structure of the compounds.

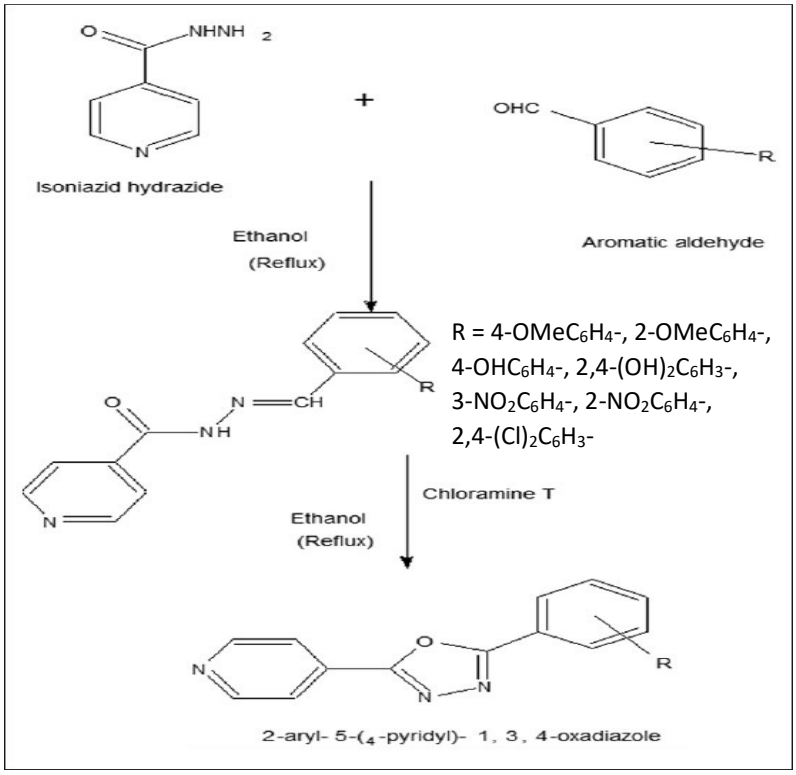


**Figure 2.8: Synthesis of 1,3,4-oxadiazoles by Biju et al.**

## 2.3 Antioxidant activity

Antioxidant activity of a compound can be evaluated by different types of in-vitro assays, such as ABTS assay, DPPH assay, Folin Ciocalteu assay and more. Among all of the choices, DPPH assay is the most commonly used method for the evaluation of antioxidant potential. Based on the research of Garcia et al. (2012), DPPH assay provides a relatively rapid and easy way for the determination of antioxidant activity by spectrophotometry, as the free DPPH radical is stable at room temperature and readily reduced by antioxidant molecules.

According to Kedare and Singh (2011), deep purple colour of DPPH solution is the result of delocalization of odd electron over the whole molecule. Upon the mixing of DPPH solution with hydrogen radical donor molecules with antioxidant potential, DPPH radical will be reduced to DPPH-H and accompanied by the colour change from deep purple to yellow.

The colour change can be measured quantitatively at the wavelength of 517 nm by using single beam UV/Vis spectrophotometer. The extent of decolourization indicates the radical scavenging ability of the antioxidant molecule. This is because the amount of purple colour DPPH radical will decrease significantly as most of the free DPPH radical is reduced in the presence of antioxidant, hence result in lower absorbance. Figure 2.9 shows the structure of free radical form and reduced form of DPPH molecules.

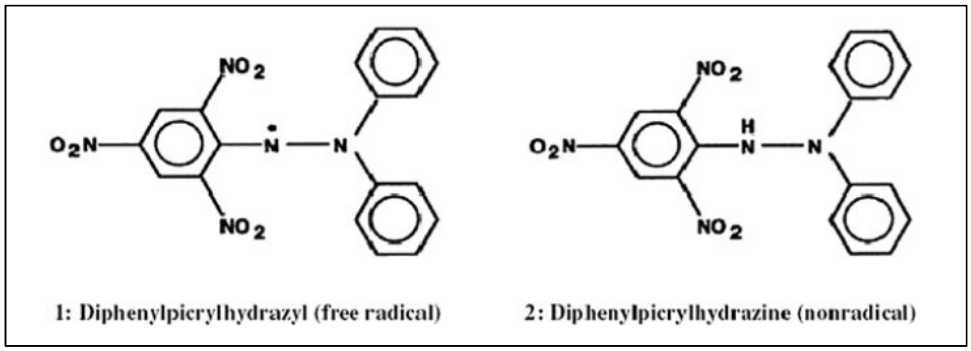


**Figure 2.9: Structure of the free radical and non-radical form of DPPH**

# CHAPTER 3

# MATERIALS AND METHODOLOGY

## 3.1 Instruments

**Table 3.7: Instruments used in the study**

| Types of instruments | Model |
|---|---|
| UV-Vis Spectrophotometer | Shimadzu UV-1280 UV-Vis spectrophotometer |
| NMR Spectrometer | FT-NMR Spectrometer JEOL JNM-ECX 400 |
| FT-IR Spectrophotometer | Perkin Elmer 2000-FTIR Spectrophotometer (Spectrum RX1) |
| Melting point apparatus | Stuart SMP10 melting point apparatus |

### 3.3.4 Purification of crude product through recrystallization

The crude solid products of carboxyl hydrazide and various 1,3,4-oxadiazole were purified by recrystallization. First, a sufficient amount of 95 % ethanol was boiled with some boiling chips and poured into the beaker containing crude products to dissolve it. The mixture was added with few boiling chips and boiled until the mixture saturated. Once all the solvent was evaporated, the dried product was rinsed with cold ethanol and the solution was sucked out. The pure solid product was dried in the oven for a few days, collected in a clean sample vial and weighed. The purity of the products was checked by using thin layer

chromatography, while the characterization of the products was carried out by using Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared Spectrophotometer (FTIR).

### 3.4 Characterization of product

#### 3.4.1 Thin layer chromatography

Thin layer chromatography is a useful technique used to monitoring the progress of the reaction and determining the purity of compounds synthesized. Thin layer chromatography requires a TLC plate as the stationary phase and solvents with different polarity as the mobile phase. A layer of silica gel was coated on the surface of aluminium foil, which is useful in separating most of the hydrocarbon compounds and amino acids. A baseline and solvent front was drawn accurately 1 cm above and below the edge of the TLC plate. The graphical representation of the TLC plate was shown in Figure 3.4.

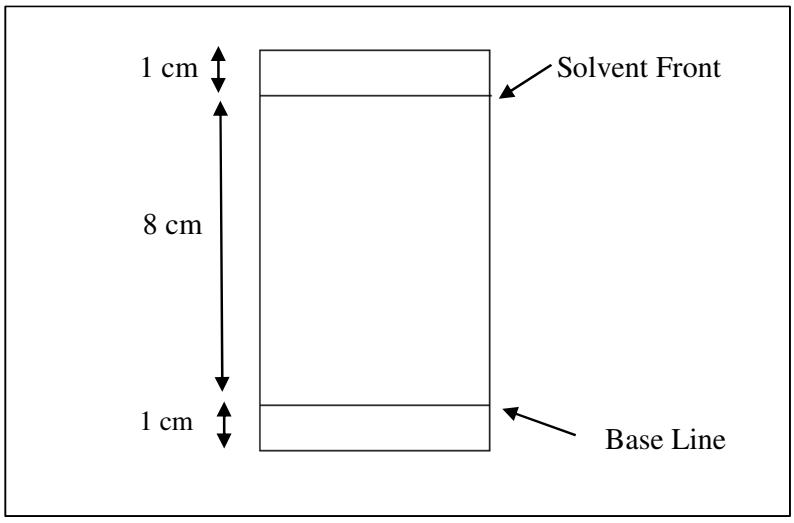


**Figure 3.4: Outline of TLC plate**

The reaction mixture or compound synthesized were diluted with a little amount of absolute ethanol and chloroform. Some of the starting materials were diluted with the same solvents for the comparison purpose later. The sample solution was spotted on the drawn baseline by using a capillary tube, with some distance between other sample spots to prevent overlapping.

The mobile phase of the TLC was prepared by mixing an equal volume of ethyl acetate and hexane to make sure the ratio is 1:1. The solvent mixture was swirled lightly to make sure the mobile phase is homogenous. The spotted TLC plate was placed in the developing chamber and the mobile phase moves over the stationary phase by means of capillary action. The TLC plate was removed once the mobile phase reached the solvent front and placed under UV lamp to visualize the separated spots.

The spot visualized under UV lamp was circled and compared with other starting materials to determine the purity of the product. The distance travelled by the mobile phase and the spotted 1,3,4-oxadiazole derivatives were measured and recorded. The distance measured was used to calculate the retention factor ($R_f$) value of the compounds.

### 3.4.2 Melting point apparatus

Melting point apparatus was used for the determination of melting point for carboxylic acid hydrazide and various 1,3,4-oxadiazole. A melting point is an important indication for the purity of the compound. A chemical compound with high purity exhibit a sharp melting point over a small temperature range, commonly around 0.5 to 1.0 °C. However, the presence of a small amount of impurities will be causing a deviation in melting point, and result in a larger melting point range. (Reddy, Khan and Nagaraja, 2016)

For the determination of melting point, a small amount of solid sample was filled inside the capillary tube with the assistance of glass funnel for sample loading. The capillary tube was then subjected to the melting point apparatus with the pre-set temperature of 200 °C, and the temperature of the melting point apparatus will slowly approach toward the temperature pre-set by the user. The melting process of the sample solid can be observed by looking into the magnifying glass. The melting point measurement was carried out twice for a more accurate determination.

### 3.4.3 Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy provide information about the functional groups present in the compound synthesized. Some of the peaks appeared in the IR

spectrum represent the presence of functional groups inside the compound. A particular functional group will only appear in certain wavenumbers or frequency range as they absorb infrared radiation at a different wavelength. Hence, by referring to the standard IR spectrum table, the identity of the functional groups present in the compounds can be determined. A typical Fourier Transform Infrared Spectrophotometer (FT-IR) operates at the frequency range between 4000 $cm^{-1}$ to 400 $cm^{-1}$.

The solid compounds were prepared in the form of KBr pellet. Initially, the solid sample was mixed with KBr pallet in the ratio of 1:10. The sample mixture was grounded into fine powder by using mortar and pestle. The fine powder containing sample was transferred into an evacuable KBr die set and pressurized to 4000 psi by using a hydraulic press. After the pressure was released, the thin KBr pallet was carefully transferred onto the sample holder for analysis. In this project, infrared spectrometry was used to determine the functional groups inside the carboxylic acid hydrazide and various 1,3,4-oxadiazole derivatives.

### 3.4.4 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectroscopy is one of the important and powerful methods to determine the molecular structure of the compound, as well as the purity of the product synthesized. The most commonly applied NMR spectroscopy are proton ($^{1}H$) NMR and carbon-13 ($^{13}C$) NMR. Proton ($^{1}H$) NMR provides information regarding the functional group of a compound based on the chemical shift, connectivity information, as well as the number of protons from the integration curve. Carbon-13 ($^{13}C$) NMR provides information more or less the same as

proton NMR, with the differences of additional chemical shift symmetry information and no proton information due to $^{1}$H-decoupling. (Soulsby and Wallner, 2016)

The irradiation frequency for both proton ($^{1}$H) NMR and carbon-13 ($^{13}$C) NMR is around 400 MHz and 100 MHz respectively. Apart from these two common NMR spectroscopy, 2D NMR spectroscopy like Distortionless Enhancement by Polarization Transfer (DEPT) analysis allow us to identify and differentiate between methine, methylene, methyl and quaternary carbons. HMBC (Heteronuclear Multiple Bond Coherence) allow us to determine the correlate long-range coupling between hydrogen and carbon atom. While Heteronuclear Multiple Quantum Coherence (HMQC) allow us to determine the connectivity between certain proton and $^{13}$C nuclei.

During the preparation of the sample, 10 mg of the samples was dissolved with an adequate amount of DMSO-$d_6$. The sample was sonicated to make sure the sample was completely dissolved. The sample was then transferred into the NMR tube by using a clean and dry dropper to prevent the possibility of contamination. The depth of the sample inside the NMR tube is around 4 cm. The NMR tube was labelled with the necessary information and send for analysis.

### 3.5 Antioxidant Activity Analysis

The antioxidant activity of carboxylic acid hydrazide and 1,3,4-oxadiazoles synthesized were determined by 2,2-diphenyl-2-picryhydrazyl (DPPH) assay. A series of sample solutions with known concentration was responsible for the

reduction of stable purple colour DPPH free radical. The extent of free radical reduction by the sample solutions was measured by single beam UV-Vis spectrophotometer. The absorbance of the sample solution was being measured at 517 nm because of the DPPH free radical shows the maximum absorption at the wavelength of 517 nm.

5 mg of sample was dissolved inside 10 mL volumetric flask with the addition of methanol to prepare a 500 ppm stock solution. A series of sample solutions with the concentration of 200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, and 6.25 ppm was prepared by serial dilution method. 1 mL of the sample solution from each concentration was transferred to a clean sample vial with cover. A standard served as a reference in the assay to compare the sample antioxidant activity against stable DPPH free radical. Butylated hydroxytoluene (BHT) was used as the standard for the antioxidant activity analysis and similar procedure have been used for the preparation of BHT standard solution.

0.1 mM of DPPH solution was prepared by dissolving 3.84 mg of DPPH in methanol inside 100 mL volumetric flask. The volumetric flask was inverted several times to make sure the DPPH solution is homogenous. The preparation of DPPH solution was carried out in a dark room and the volumetric flask was covered with aluminium foil due to high photosensitivity of DPPH solution. In the preparation of the sample for the absorbance measurement, sample solution with different concentration was added with 4 mL of DPPH solution and shake vigorously to mix homogeneously. A blank solution was prepared in the same

way by replacing 1 mL of the sample solution with methanol. These sample solutions were covered with aluminium foil and incubated in dark condition for 30 minutes.

The absorbance of the sample solution was measured by using single beam UV/Vis spectrophotometer at the wavelength of 517 nm. The absorbance obtained was used to calculate the percentage of radical scavenging in order to determine $IC_{50}$. $IC_{50}$ is known as an effective concentration to reduce 50% of DPPH free radical after 30 minutes, it can be determined by using the graph of the percentage of radical scavenging against the concentration of the sample solution.

### 3.5 Calculation

To determine the mass of starting materials required for the synthesis.

$$\text{Mass} = \text{Molar mass } (\text{gmol}^{-1}) \times \text{Number of mole required (mol)}$$

To determine the volume of starting materials in liquid form required for the synthesis.

$$\text{Volume} = \text{Mass (g)} \times \text{Density of the starting material } (\text{gmL}^{-1})$$

For the determination of percentage yield for the product synthesized.

$$\text{Percentage yield} = \frac{Experimental\ mass\ of\ the\ product}{Theoretical\ mass\ of\ the\ prodict} \times 100\%$$

For the determination of the retention factor ($R_f$) value.

Retention factor ($R_f$) = $\frac{Distance\ travelled\ by\ the\ sample\ component\ from\ the\ baseline}{Distance\ travelled\ by\ the\ solvent\ from\ the\ baseline}$

For the determination of $IC_{50.}$

Percentage (%) radical scavenging = $\frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100\ \%$

Where $A_{blank}$ = absorbance of blank after 30 minutes incubation

$A_{sample}$ = absorbance of sample after 30 minutes incubation

# CHAPTER 5

# CONCLUSION

## 5.1 Conclusion

In this project, carboxylic acid hydrazide was synthesized for the synthesis of 1,3,4-oxadiazoles. Four new 1,3,4-oxadiazoles bearing an indole ring was synthesized successfully by the reaction between carboxylic acid hydrazide and various 4-substituted benzoic acid, in the presence of concentrated phosphorus oxychloride. The structure of carboxylic acid hydrazide and 1,3,4-oxadiazole bearing an indole ring were elucidated and characterized by using spectroscopic methods including FT-IR, $^{1}$H NMR, $^{13}$C NMR, DEPT, HMQC, and HMBC.

Antioxidant activity of carboxylic acid hydrazide and 1,3,4-oxadiazoles bearing an indole ring was determined through in-vitro DPPH assay, by using BHT as the standard reference. All of the compounds tested against DPPH assay giving an $IC_{50}$ value of more than 200 ppm, indicating that the carboxylic acid hydrazide and 1,3,4-oxadiazoles exhibit a weak antioxidant activity compared to the standard with the $IC_{50}$ value of 31 ppm.

## 5.2 Further study

Several articles and journals have reported that 1,3,4-oxadiazole possess a wide range of biological and pharmacological activities. Hence further study on antibacterial activity, anticancer activity, antifungal activity, or anti-

inflammatory activity can be carried out on the 1,3,4-oxadiazole synthesized. Apart from that, different 1,3,4-oxadiazole can be synthesized with some structural modification by reaction between carboxylic acid hydrazide with various substituted benzoic acid as starting materials.

## REFERENCES


Alam, M., Bristi, N. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), pp. 143-152.

Bala, S., Kamboj, S., Kajal, A., Saini, V. and Prasad, D. (2014). 1,3,4-Oxadiazole derivatives: Synthesis, characterization, antimicrobial potential, and computational Studies. *BioMed Research International*, 2014, pp. 1-18.

Barbuceanu, S., Ilies, D., Saramet, G., Uivarosi, V., Draghici, C. and Radulescu, V. (2014). Synthesis and antioxidant activity evaluation of new compounds from hydrazinecarbothioamide and 1,2,4-triazole class containing diarylsulfone and 2,4-difluorophenyl moieties. *International Journal of Molecular Sciences*, 15(6), pp. 10908-10925.

Biju, C., Ilango, K., Prathap, M. and Rekha, K. (2012). Design and microwave-assisted synthesis of 1,3,4-oxadiazole derivatives for analgesie and anti-inflammatory activity. *Journal of Young Pharmacists*, 4(1), pp. 33-37.

Gadegoni, H. and Manda, S. (2013). Synthesis and screening of some novel substituted indoles contained 1,3,4-oxadiazole and 1,2,4-triazole moiety. *Chinese Chemical Letters*, 24(2), pp. 127-130.

Garcia, E., Oldoni, T., Alencar, S., Reis, A., Loguercio, A. and Grande, R. (2012). Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Brazilian Dental Journal*, 23(1), pp. 22-27.

Hossain, M., Brunton, N., Barry-Ryan, C., Martin-Diana, A. and Wilkinson, M. (2008). Antioxidant activity of spice extracts and phenolics in comparison to synthetic antioxidants. *Rasayan Journal of Chemistry*, 1(4), pp. 751-756.

Inman, M. and Moody, C. (2013). Indole synthesis – something old, something new. *Chemical Science*, 4(1), pp. 29-41.

Iyer, V., Gurupadayya, B., Koganti, V., Inturi, B. and Chandan, R. (2016). Design, synthesis and biological evaluation of 1,3,4-oxadiazoles as promising anti-inflammatory agents. *Medicinal Chemistry Research*, 26(1), pp. 190-204.

Jha, K., Samad, A., Kumar, Y., Shaharyar, M., Khosa, R., Jain, J., Kumar, V. and Singh, P. (2010). Design, synthesis and biological evaluation of 1,3,4-oxadiazole derivatives. *European Journal of Medicinal Chemistry*, 45(11), pp. 4963-4967.

Kaur, R. and Kaur, P. (2018). *Synthesis and pharmacological activities of 1,3,4-oxadiazole derivatives: A review*. [online] Research Gate. Available at: https://www.researchgate.net/publication/327867959_SYNTHESIS_AND_PHARMACOLOGICAL_ACTIVITIES_OF_134-OXADIAZOLE_DERIVATIVES_A_REVIEW [Accessed 6 Mar. 2019].

Kedare, S. and Singh, R. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4), pp. 412-422.

Kumar Kolli, S. (2016). Synthesis, characterization and biological activity of some new of novel 1,3,4-oxadiazole bearing thiophen-2-carboxylic Acid. *World Journal Of Pharmacy And Pharmaceutical Sciences*, 5(5), pp. 1800-1825.

Majumdar, P., Pati, A., Patra, M., Behera, R. and Behera, A. (2014). Acid hydrazides, potent reagents for synthesis of oxygen-, nitrogen-, and/or sulfur-containing heterocyclic rings. *Chemical Reviews*, 114(5), pp. 2942-2977.

Megally Abdo, N. and Kamel, M. (2015). Synthesis and anticancer evaluation of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and mannich bases. *Chemical and Pharmaceutical Bulletin*, 63(5), pp. 369-376.

Mickevičius, V., Vaickelionienė, R. and Sapijanskaitė, B. (2009). Synthesis of substituted 1,3,4-oxadiazole derivatives. *Chemistry of Heterocyclic Compounds*, 45(2), pp. 215-218.

Modi, V. and Modi, P. (2012). Oxadiazole: Synthesis, characterization and biological activities. *Journal of Saudi Chemical Society*, 16(3), pp. 327-332.

Patel, K., Prajapati, S., Panchal, S. and Patel, H. (2014). Review of synthesis of 1,3,4-oxadiazole derivatives. *Synthetic Communications*, 44(13), pp. 1859-1875.

Reddy, C., Khan, K. and Nagaraja, C. (2016). A review on the determination of melting point measurement system. *International Journal of Advanced Research in Electrical, Electronics and Instrumentation Engineering*, 5(2), pp. 975-979.

Saha, A., Kumar, R., Kumar, R. and Devarkumar, C. (2010). Development and assessment of green synthesis of hydrazides. *Indian Journal of Chemistry*, 49B, pp. 526-531.

Siddalingamurthy, E. (2014). *Studies on development of new protocol for the synthesis of indole heterocycles*. [online] Hdl.handle.net. Available at: http://hdl.handle.net/10603/144846 [Accessed 4 Apr. 2019].

Soulsby, D. and Wallner, A. (2016). Introduction to NMR spectroscopy in the undergraduate curriculum. *ACS Symposium Series*, pp. 1-10.