SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLES INCORPORATING AN INDOLE MOIETY

By

CHONG JIEN LEE

A project report submitted to Department of Chemical Science

Faculty of Science

University Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Chemistry

May 2019

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ABSTRACT

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CHONG JIEN LEE

In this project, an indole ester, carboxyl hydrazide and four new 1,3,4-oxadiazoles derivatives have been successfully synthesized and characterized. The four new 1,3,4-oxadiazoles derivatives were synthesized by the reaction of carboxyl hydrazide and benzoic acid derivatives which being named as **JL1**, **JL2 JL3** and **JL4** respectively. The structures of carboxyl hydrazide and 1,3,4-oxadiazole derivatives (**JL1-JL4**) were characterized by ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, and IR spectroscopic. The antioxidant activity of carboxyl hydrazide and 1,3,4-oxadiazole derivatives were evaluated by using 2,2-diphenyl-2-picryhydrazyl hydrate (DDPH) radical scavenging assay where the carboxyl hydrazide and 1,3,4-oxadiazole showed weak antioxidant activity.

ABSTRAK

SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLES INCORPORATING AN INDOLE MOIETY

CHONG JIEN LEE

Dalam kajian ini, satu indole ester, carboxyl hidrazide dan empat 1,3,4-oxadiazoles derivatif baru telah disintesiskan dan dicirikan. Empat 1,3,4-oxadiazoles derivatif merupakan sintesis daripada reacksi antara carboxyl hidrazide dan benzoic asid derivatif yang dinamakan sebagai **JL1**, **JL2**, **JL3** dan **JL4**. Struktur carboxyl hidrazide dan semua 1,3,4-oxadiazole **JL1-JL4** telah dicirikan dengan menggunakan ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC dan IR spektroskopi teknik. Aktiviti antioksidan bagi carboxyl hidrazide dan 1,3,4-oxadiazole derivatif telah ditentukan dengan mengguankan 2,2-diphenyl-2-picryhydrazyl hydrate (DDPH) radikal pemerangkap cerakin tetapi carboxyl hidrazide dan 1,3,4-oxadiazole menunjuk aktiviti antioksidan yang lemah.

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The completion of my research paper is never the work of alone. The contributions from numerous people in their different ways have made it possible. Firstly, my heartfelt gratitude goes out to my final year project supervisor, Associate Professor Dr. SIM KOOI MOW for his keen observation regarding my work, providing valuable insights, guidance and patience throughout the project period.

Besides that, I would like to thank my parent for their encouragement and supportiveness throughout this project. Then I would also like to thank the laboratory staff that helped me throughout this project, as well as the lecturers who taught me the basic knowledge in Chemistry.

Last but not least, my sincere and specially thanks to my teammates, Kong Kian Liang and Fen Ju Ni as well as my others beloved friends for their encouragement, companion and comments throughout this project.

DECLARATION

I hereby declare that the thesis is based on my original work except quotations and citations which have been duly acknowledged. I also declare that is has not been previously and concurrently submitted for any degree at University Tunku Abdul Rahman or other institutions.

Name: Chong Jien Lee

Date:

APPROVAL SHEET

This thesis entitled "**SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLES INCORPORATING AN INDOLE MOIETY**" was prepared by CHONG JIEN LEE and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (HONS) Chemistry at Universiti Tunku Abdul Rahman.

Approved by,

Supervisor

Date:

(DR SIM KOOI MOW)

Associate Professor

Department of Chemical Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UUNIVESITY TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **CHONG JIEN LEE** (ID No: **15ADB05589**) has completed this thesis entitled "SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,3,4-OXADIAZOLES INCORPORATING AN INDOLE MOIETY" under the supervision of **ASSOCIATE PROFESSOR DR. SIM KOOI MOW** from the Department of Chemical Science.

I hereby give permission to my supervisor to write and prepared manuscripts of these research findings for publishing in any form, if I did no prepare it within six months' time from this date, provided that my name is included as one of the authors for the articles. Arrangement of names will depend on my supervisor.

Your truly,

_____

(CHONG JIEN LEE)

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LIST OF ABBREVIATIONS

%	percentage
% v/v	percentage concentration volume per volume
% v/w	percentage concentration volume per weight
℃	Celcius
δ	chemical shift (ppm)
^{1}H	Proton
^{13}C	Carbon-13
BHT	butylated hydroxytoluene
D_2O	deuterated oxide
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EA	ethyl acetate
EtOH	ethanol
FTIR	Fourier Transform Infrared
g	gram
H_2O	water
HCl	hydrochloric acid
H_2SO_4	sulphuric acid
HMBC	Heteronuclear Multiple Bond Coherence

HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
JL	1,3,4-oxadiazole
mg	milligram
mL	millilitre
mM	millimolar
mmol	millimole
mol	mole
nm	nanometer
NMR	Nuclear Magnetic Resonance
$POCl_3$	phosphoryl chloride
ppm	parts per million
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 Indole

Indole is a benzene ring that fused to a five-membered heterocylic ring which containing a nitrogen atom. It was initially found by Adolph Baeyer as a consequence of his research on the structure of indigo. In his research, two different compounds such as C_8H_7NO and $C_8H_7NO_2$ was obtained as the reduction products of isatin then he regarded them as the oxygen derivatives of a hypothetical parent, C_8H_7N which being named as indole. The two compounds were named as oxindoles and dioxindoles. The structure of indole was being confirmed when he managed to reduce oxindole to indole through the distillation of a mixture of oxindole and zinc dust (Roussel, 1953). In other words, indole was named after indigo and oleum as it was being prepared and identified from the reaction of the indigo dye with oleum. Figure 1.1 shows the structure of indole.

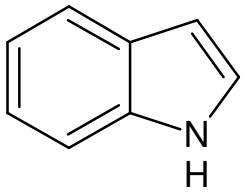
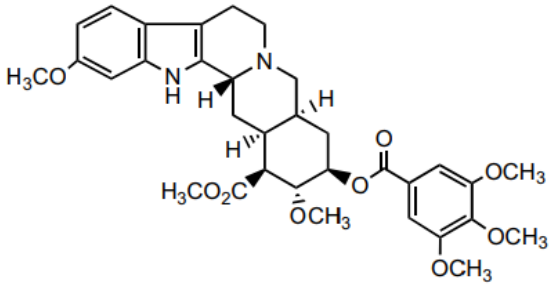
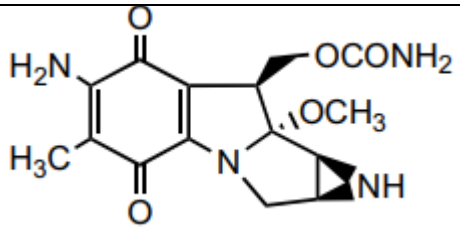
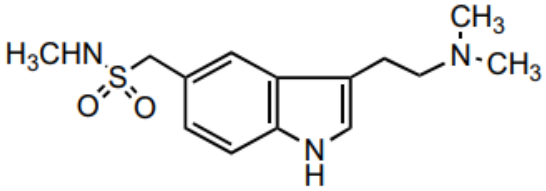
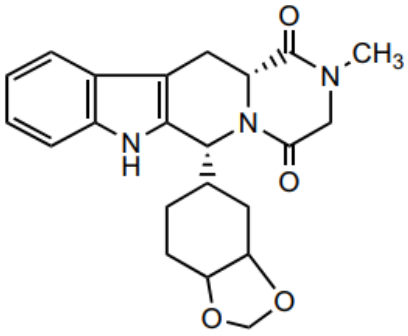


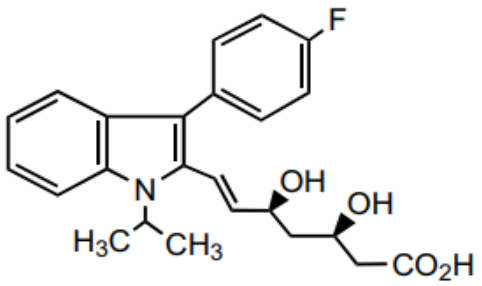
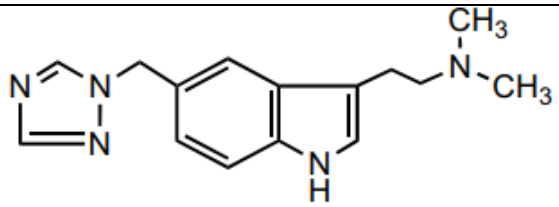
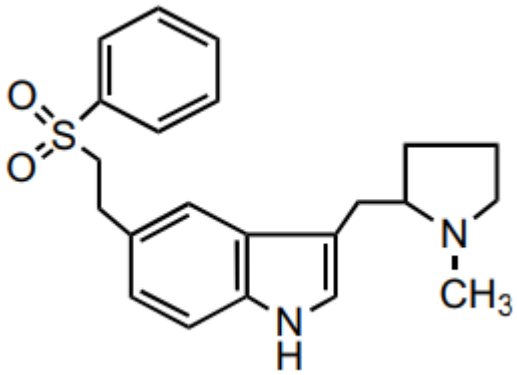
Figure 1.1 Structure of indole

There are many naturally occurring substances which possess the indole ring as a parent. For instance, the ancient dye (indigo), the essential amino acid (tryptophan), the plant hormone (heteroauxin), vasoconstrictor and serotonin. Indole, itself can be isolated from certain plant sources, especially form the jasmine and certain citrus fruits which using the method of degradation of product of some higher derivative. (Roussel, 1953)

Indole is an important functional group in the structures of different dyes, fragrances, pharmaceuticals and agricultural chemicals. The importance of indole ring moieties in diverse natural pharmaceutical agents had made their synthesis and functionalization became the key field in synthetic organic chemistry (Heravi et al., 2017). The pharmaceutical activities that indole ring exhibit are antihistaminic, antifungal, antimicrobial, antioxidant, plant growth regulator, anti-HIV, anticonvulsant, anti-inflammatory, analgesic and etc. Table 1.1 shows the indole based drugs available in clinical uses.

Table 1.1 Indole based drugs available in clinical uses

Drug	Structure	Functions
Reserpine		Tranquilizer
Mitomycin		Cancer chemotherapy
Sumatriptan		Antimalarial
Tadalafil		Antidiabetic

Rizatriptan		Anti-tubercular
Fluvastatin		Anti-leishmanial
Eletriptan		Anti-convulsant

(Singh and Singh, 2018)

1.1.1 Fisher Indole Synthesis

Due to various importance of indole ring moieties in pharmaceutical uses, different methods and techniques have been established to synthesize the indole ring moieties. Among the methods, Fischer Indole Synthesis (FIS) is the one which is

well-known and common techniques which used to synthesis indole. (Heravi et al., 2017).

Fisher Indole Synthesis (FIS) is developed by the Emil Fischer and Friedrich Jourdan in 1883 in which it is a method for preparation of substituted indoles. In FIS, the use of acid catalyst is very critical. Brønsted acids such as hydrochloric acid, HCl and sulphuric acid, H_2SO_4 were frequently used effectively in the reaction while Lewis acids such zinc (II) chloride, $ZnCl_2$, iron (III) chloride, $FeCl_3$, and aluminium chloride, $AlCl_3$ are also favorable catalysts for the reaction (Heravi et al., 2017).

It is a reaction where the ammonia is eliminated from the aryl hydrazone of an aldehyde or ketone, by treatment with acid or various metal and anhydrous metal salt catalysts, with formation of an indole nucleus (Dobbs et al., 2014). In other words, Fisher Indole synthesis is the reaction between an aryl hydrazone with either aldehyde or ketone in the presence of acid as catalyst such as Bronsted acids and Lewis acids, at elevated temperature. With the presence of catalyst, heating on aryl hydrazone leads to the elimination of ammonia and formation of indole. Figure 1.2 shows the general reaction of Fisher Indole Synthesis.

Figure 1.2 General reaction of Fisher Indole Synthesis

1.2 Hydrazine and hydrazide

Hydrazine is a colorless, oily liquid with an ammonia-like odor, which has the molecular formula of N_2H_4. It is a hazardous chemical compound as in short-term exposure of high level of hydrazine, human may have the symptoms such as irritation of the eyes, nose, and throat, dizziness, headache, nausea, seizures, and even coma as well as bring damage to the liver, kidneys, and central nervous system in humans. The liquid of hydrazine is corrosive and may produce dermatitis from skin contact in humans and animals. Effects to the lungs, liver, spleen, and thyroid have been reported in animals if frequently exposed to hydrazine via inhalation.

Hydrazine, itself has been widely used either in laboratory or in industrial. It was being used in water waste treatment by removing the halogens or acting as boiler in water treatment. Besides, hydrazine also being used as reducing agent in nickel plating, a chain extender in the polymerization of polyurethane, a rocket propellant as well as an intermediate in industrial synthetic chemistry. In the perspective of agriculture, it has been used to synthesis agricultural chemical such as maleic hydrazide and used in cultivation of tobacco as well as in potato and onion storage (Timperio, Rinalducci and Zolla, 2005).

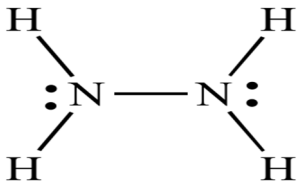


Figure 1.3 Structure of hydrazine

In biological perspectives, hydrazine and its derivatives can be used in medicinal chemistry. Hydrazides, a related class of compounds of hydrazine, which show interest in biological activity and also pharmacological activity due to the functional groups of $NHNH_2$ and NHN=CH- groups with the availability of proton (Khan, Siddiqui and Tarannum, 2017). Table 1.2 shows the examples of hydrazine derivatives and their explanation.

Table 1.2 Examples of hydrazine derivatives and their explanation

Types of hydrazine derivatives	**Explanation**
Hydralazine	It is an anti-hypertensive and peripheral vasodilator drug that manage the high blood pressure. Recently, it has gained interest in treatment of cancer due to it prevents the transfer of methyl group to DNA in several cancer-silencing or tumor suppressor genes through the inhibition of DNA methyltransferases I. However, hydralazine can cause damage of DNA and it is being testified to cause some incidence of lung tumors in mice.
Isoniazid	It is an anti-tuberculosis drug, however, it is toxic, may cause a severe hepatotoxicity, and also lead to liver cancer.

Iproniazid	It is a monoamine oxidase inhibitor that been used as an antidepressant. Yet, it has been prohibited in medical use as it also causing severe hepatotoxicity in humans.
Procarbazine	It is an anticancer drug that used in the treatment of Hodgkin's lymphoma, malignant melanoma and brain tumors in children. It is mutagenic for bacterial and mammals as well as carcinogenic to mice, rats and monkeys

(Sinha and Mason, 2014)

In previous studies, it is show that different substituted hydrazides and their derivatives possess potential biological activity which range from anticonvulsant, antidepressant, analgesic, anti-inflammatory, antimalarial, antimicrobial, anticonvulsant, anticancer, vasodilator, antiviral, anti-HIV, anthelmintic, antidiabetic, and etc. (Khan, Siddiqui and Tarannum, 2017)

The hydrazide moiety has been used as active functional group in organic synthesis. For instances, Ugi multicomponent reactions, Ugi-azide multicomponent reactions, the synthesis of spiroquinazolinones, and the preparation of tetrazoles as well as acting as organocatalysts in chemical syntheses. However, it was become a problematic issue when hydrazides were used as starting materials. This is because of the presence of the regioselectivity between the two competitive amines present in its structure which are the N(1) and N(2). In previous study, the reactivity through the N(1) has been found to participate in cross-coupling reactions as well

as in Michael addition reactions, while the reactivity through the N(2) has been restricted to the synthesis of hydrazones (Ziarani and Vavsari, 2017).

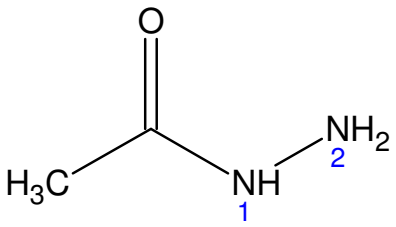


Figure 1.4 Structure of hydrazide

1.3 Oxadiazoles

Oxadiazole is an aromatic heterocyclic compound with the molecular formula of $C_2H_2N_2O$. It is a five-membered ring compound which consists of an oxygen and two nitrogen atoms. Oxadiazole was first discovered in 1884 by Tiemann and Krüger with the name of furo[ab]diazoles. It can be considered as the resultant from the furan by replacing two methane (–CH=) groups by two pyridine type nitrogen atoms (–N=). This replacement as result in the reduction of their aromaticity in order that some of their isomers are electronically comparable to conjugated diene systems (Pitasse-Santos, Sueth-Santiago and Lima, 2018). Different regioisomeric forms are existed for oxadiazoles which can be differentiated into four major types: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, and 1,3,4-oxadiazole (Patel et al., 2014).

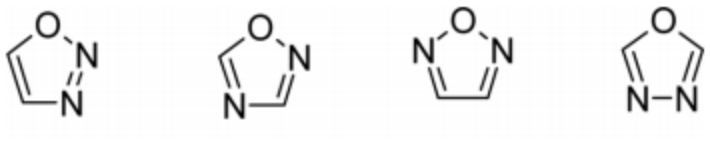


Figure 1.5 Regioisomeric forms of oxadiazoles

Among the four isomers, 1,2,4-oxadiazole and 1,3,4-oxadiazole are well known by the researchers because of their various application. 1,3,4-oxadiazole derivatives are the most stable isomer among the four whereas 1,2,3-oxadiazole derivatives are quite unstable and it will revert in the form of diazoketone tautomer (Palit, Saraswat and Sahoo, 2016).

Oxadiazole cannot undergo electrophilic substitution reactions due to the low density of electrons on the carbon atom that causes the electron withdrawal effect of pyridine type nitrogen when there is electron releasing group is added to it. It is also found that oxadiazole also resist to nucleophilic substitution reactions. However, halogen substituted oxadiazole can undergo the nucleophilic substitution reactions by replacing the halogen a nucleophile (Palit, Saraswat and Sahoo, 2016).

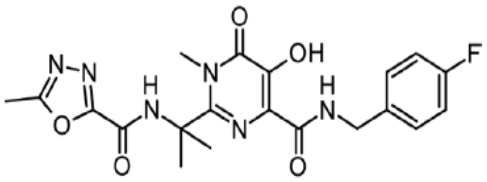
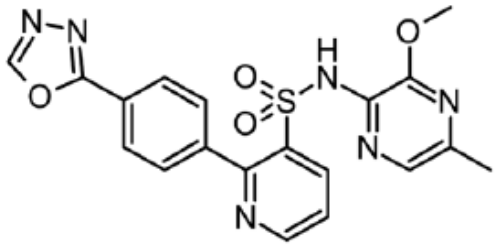
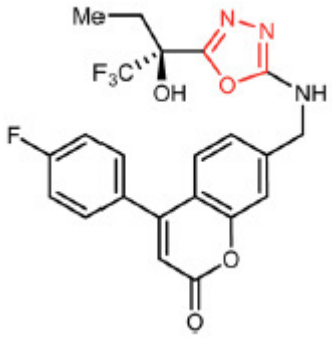
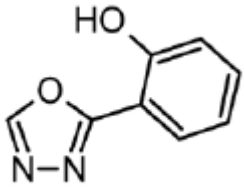
The oxadiazoles have gained interest in the medical filed due to their biological and pharmaceutical activities such as antimicrobial, antiflammatory, antifungal, antitubercular, anticonvulsant, anthelmintic, herbicidal, antioxidant, analgesic, antitumor and antihepatitis B viral activities (Palit, Saraswat and Sahoo, 2016). Other than medical field, oxadiazole moieties also can be applied in several areas, for examples, as luminescent materials, electron-transport materials, polymers,

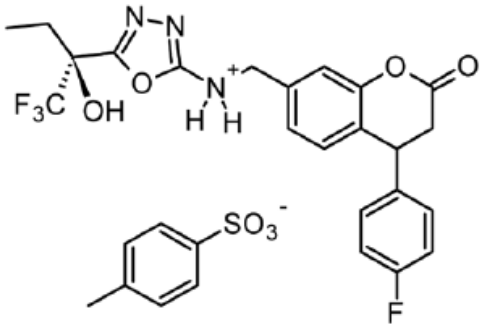
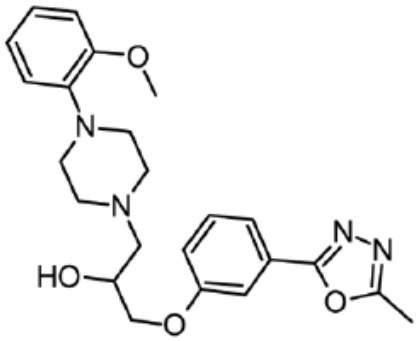
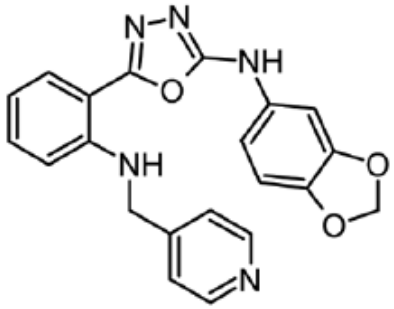
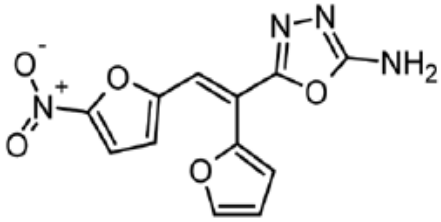
herbicides, and corrosion inhibitors (Pitasse-Santos, Sueth-Santiago and Lima, 2018).

1.3.1 1,3,4-oxadiazoles

In 1965, 1,3,4-oxadiazole was initially discovered by Ainsworth through the thermolysis of ethylformate, formally hydrazine, at atmospheric pressure. The common names of 1,3,4-oxadiazole are oxybiazole, diazoxole, furo(bb')diazole, and biozole and later those common names are being changed by the IUPAC name of 1,3,4-oxadiazole (Patel et al., 2014). Many studies have showed that 1,3,4-oxadiazole have been exploited for various application. For examples, it is being applied in the development of advanced materials, such as electroluminescent and electron-transport materials as well as the polymer and material science applications (Tokumaru and Johnston, 2017). Besides that, 1,3,4-oxadiazoles also exhibit the biological and pharmaceutical activities such as antibacterial, antifungal, anti-inflammatory, analgesic, anticonvulsant, antihypoglycemic and insecticidal properties. Some of these compounds have also anticancer, anti-HIV agent, antiparkinsonian and antipriliferative agent (Kolli, 2016). Table 1.3 shows the summary of common 1,3,4-oxadiazoles derivatives and their function respectively.

Table 1.3: Summary of common 1,3,4-oxadiazoles derivatives and their function

Name	Structure	Function
Raltegravir		Antiretroviral drug
Zibotentan		Anticancer agent
Setileuton		Anti-infammatory agent
Fenadiazole		Hypnotic drug

MK-0633 p-toluenesulfonate		5-lypoxynase inhibitor
Nesapidil		Antihypertensive agent
ABT-751-oxadiazole		Antibiotics
Furamizole		Antibiotics

(Patel et al., 2014)

(Tokumaru and Johnston, 2017)

1.4 Antioxidant activity

In biological system, free radicals are produced inevitably and encountered exogenously, especially the oxygen derived free radicals. Free radicals are atoms, molecules or ion with unpaired electron and they are reactive to the chemical reactions with other molecules. Free radicals that derived from the oxygen are known as reactive oxygen species (ROS). The example of these free radicals are superoxide anion ($O_2^{\bullet}$), perhydroxyl radical ($HO_2^{\bullet}$), hydroxylradical ($^{\cdot}OH$), nitric oxide and etc. In biological system, ROS are formed during cellular metabolism and functional activities which have the function in cell signaling, apopotosis, ion transportation and gene expression (Lü et al., 2010).

However, excessive amount of ROS can cause the deleterious effect, for example oxidative stress. Oxidative stress is where there is lack of balance between the ROS and the organism's ability to counteract their action by the antioxidative protective system. Oxidative stress has been proved as a contributor to the pathogenesis and pathophysiology of many chronic health problems, cardiovascular and inflammatory diseases and cancer. Besides, it also has negatively influence to the biology of aging, impairment of physiological functions, promoting diseases incidence and reducing life span (Pisoschi and Pop, 2015).

To avoid the stated abnormalities occurred, antioxidant has been used. Antioxidants are the molecules that can neutralize free radicals by donating or accepting the

electron to eliminate the unpaired electrons. In other words, antioxidants are able to delay or inhibit cellular damage to the human body. Besides, they are being used for stabilization of polymeric products, of petrochemical, foodstuffs, and cosmetics (Pisoschi and Negulescu, 2011).

Antioxidants can be obtained naturally or synthetically. Natural antioxidants are mainly extracted from plants such as fruits, vegetables, spices, grains and herbs. This is due to the presence of phenolics such as phenol and polyphenols, flavonoids, carotenoids, steroids and thiol compounds which are the antioxidant compounds. They can help to minimize the cellular damaged due to oxidative stress and also reduce the risk of chronic diseases. Tert-butylhydroxyl-toluene, tert-butylhydroxyanisole and tert-butylhydroquinone are the common synthetic antioxidants that have been widely used especially in food industry. However, these antioxidant are not suitable to be used in pharmacological because of toxicological and carcinogenic concerns (Lü et al., 2010)

1.5 Objectives

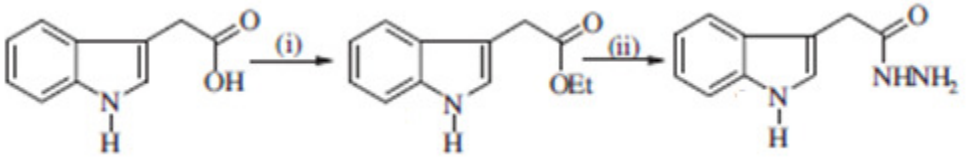
1. To synthesize an indole ester.
2. To synthesize a carboxyl hydrazide bearing indole ring.
3. To synthesize a series of new 1,3,4-oxadiazoles from carboxylic hydrazide and benzoic acid dericatives.
4. To characterize carboxylic hydrazide and 1,3,4-oxadiazoles by ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, FTIR, and melting point apparatus.
5. To carry out antioxidant activity of carboxyl hydrazide and 1,3,4-oxadiazoles by using DPPH assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of hydrazides

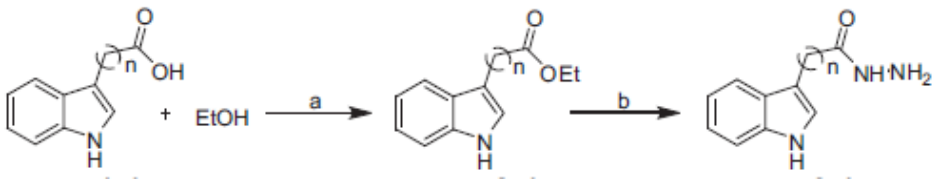
According to Gadegoni and Manda (2013), synthesis of (1H-indol-3-yl)-acetic acid hydrazide can be done by reacting (1H-indol-3-yl)-acetic acid ethyl ester and hydrazine hydrate. Before that, Gadegoni and Manda (2013) was carried out the reaction between indole-3-acetic acid (0.01 mol) and absolute ethyl alcohol (25 mL) in the presence of concentrated H_2SO_4 (2 mL) to synthesis the (1H-indol-3-yl)-acetic acid ethyl ester. The ester was synthesized under reflux for two hours and then was poured into the ice cold water after completion of reaction. The crude product was obtained after being filtered, washed with 10% $NaHCO_3$ solution and dried. Recrystallization from ethyl alcohol was done to obtain the pure ester. The synthesized ester (0.01 mol) was then undergo refluxed with hydrazine hydrate (0.025 mol) in ethanol (20 mL) for 7 hours to obtain the hydrazide compound. the product was cooled to room temperature, filtered and recrystallized from ethanol to obtain the (1H-indol-3-yl)-acetic acid hydrazide. The reaction for synthesis of (1H-indol-3-yl)-acetic acid hydrazide shown in Figure 2.1.



(i): EtOH, H_2SO_4, 2 hours (ii): N_2H_4, EtOH, 7 hours

Figure 2.1: Synthesis of (1H-indol-3-yl)-acetic acid hydrazide

Rapolu et al. (2013) reported a series of reaction to synthesis indole-3-carboxylic acid hydrazides. First and foremost, esterification of 10 mmol of indole-3-carboxylic acids with ethanol was done in the presence of concentrated H_2SO_4 was done to obtain indole-3-carboxylates. The reaction was under reflux for 3 to 4 hours and then the mixture was cooled and solvent ethanol was removed under vacuum before being poured out into the ice. The reaction mixture is then treated with 10% of aqueous sodium hydroxide solution till it became slightly basic. The pure indole-3-carboxylates was obtained after filtered, washed with water and dried. Next, the synthesized indole-3-carboxylates was used to synthesis indole-3-carboxylic acid hydrazides by reacting with hydrazine hydrate (15 mL, 99%) in the solvent such as ethanol (15 mL). After refluxed for 5 to 6 hours, the reaction mixture was being cooled and poured into ice. Extraction with ethyl acetate was done three times and the organic layer was collected and dried over anhydrous sodium sulphate and concentrated under vacuum to yield the product. Figure 2.2 shows the scheme reaction of synthesis indole-3-carboxylic acid hydrazides.



a: EtOH, H_2SO_4, 3-4 hours b: N_2H_4, EtOH, 5-6 hours

Figure 2.2: Synthesis indole-3-carboxylic acid hydrazides

Saha et al. (2010) had reported that there were two methods to synthesize carboxylic acid hydrazides in which one of the methods was in conventional while another was in green synthesis. For the conventional method, ester of carboxylic acid was synthesis at first by refluxing 0.0246 mol of carboxylic acid with 0.25 mol of absolute ethanol and 0.5 g of concentrated sulphuric acid for 3 to 4 hours. The reaction mixture was cooled down after the reaction was completed while the excess ethanol was evaporated on a water bath. The reaction mixture was the extracted with carbon tetrachloride followed by sodium hydrogen carbonate to remove the acid and washed with water. Magnesium sulphate is used to dry over the organic layer then the layer was filtered and distilled to obtain the ester of carboxylic acid. Next, the synthesized ester (0.01 mol) and hydrazine hydrate (0.011 mol) was dissolved in ethyl alcohol and refluxed for 3 to 5 hours. Once the reaction was completed, the mixture was concentrated under vacuum to distill off the ethanol to obtain the desired product. Pure hydrazide compound was obtained after being recrystallized from the alcohol.

Besides that, Saha et al. (2010) also reported the green synthesis of carboxylic acid hydrazide. 0.01 mol of carboxylic acid and 0.012 mol of hydrazine hydrate were mixed together in conical flask. Then the reaction mixture was irradiated under microwave for 60 to 200 seconds at 900 Watt at 2.45 GHz. After that, the reaction mixture was cooled to -20 ℃ and then lyophilized at -50 ℃. The carboxylic acid hydrazide was obtained in pure after recrystallized from methyl alcohol. Figure 2.3 shows the synthesis of carboxylic acid hydrazide by using microwave irradiation.

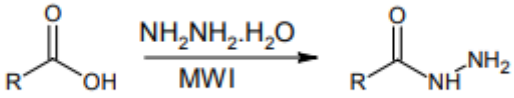


Figure 2.3: Synthesis of carboxylic acid hydrazide by using microwave irradiation

Abdel Hamid et al. (2004) described a series of reaction for synthesis the aryloxyacetic acid hydrazides by using microwave irradiation. First of all, aryloxyacetic acids were synthesized from the reaction of phenolic compounds and sodium hydroxide in water and treated with chloroacetic acid and bentonite. The reaction mixture was irradiated in the microwave oven for 5 minutes then extracted from the paste in the minimum amount of water. It was then filtered, washed, acidified with sulphuric acid and recrystallized from hot water. Secondly, methyl aryloxyacetates was prepared from the reaction of aryloxyacetic acids, methyl alcohol and concentrated sulfuric acid which was irradiated in microwave oven for 2 minutes. The mixture was then cooled, neutralized with sodium bicarbonate solution and filtered to give the products. Lastly, formation of aryloxyacetic acid

hydrazides was done by reacting 0.001 mol of methyl ester in 5 mL of methyl alcohol with 0.01 mol of hydrazine hydrate in microwave irradiation for one minut and recrystallized from ethanol. Figure 2.4 shows the scheme reaction for synthesis the aryloxyacetic acid hydrazides.

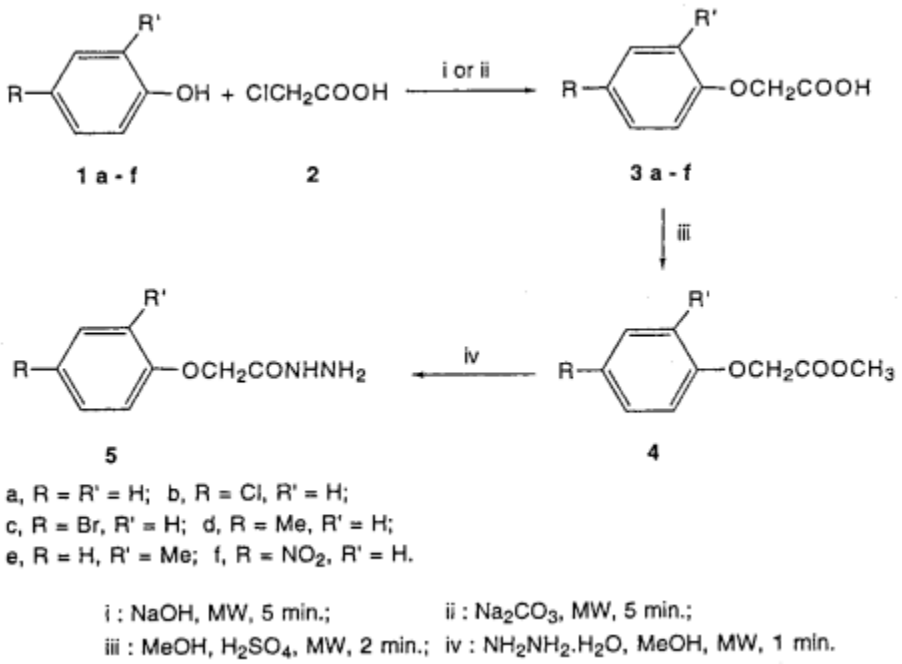


Figure 2.4: Reaction for synthesis the aryloxyacetic acid hydrazides

Cihan-Üstündağ et al. (2016) stated that 5-fluoro-3-phenyl-1H-indole-2-carbohydrazide can be synthesized from a series of reaction. Firstly, solution of 4-fluoroaniline was reacted with ethanol, water, concentrated hydrochloric acid and 7% aqueous $NaNO_2$ solution to produce diazonium salt. The diazonium salt was then reacted with ethyl 2-benzyl-3-oxo-butanoate, ethanol, water and potassium hydroxide to synthesis ethyl 2-benzyl-2-(4-fluorophenylhydrazono) acetate. Next, ethyl 5-fluoro-3-phenyl-1H-indole-2-carboxylate was synthesized from the

reaction of ethyl 2-benzyl-2-(4-fluorophenylhydrazono) acetate and concentrated hydrochloric acid under reflux for 4 hours. Then, the 0.02 mol of the crude product that synthesized was added to the mixture solution with 20 mL of ethanol and 8 mL of 98 % hydrazine hydrate. The reaction mixture was refluxed for 6 hours. Once the reaction completed, the resulting brown crystals were filtered off and recrystallized from ethanol and chloroform. Figure 2.5 shows the synthesis reaction of 5-fluoro-3-phenyl-1H-indole-2-carbohydrazide.

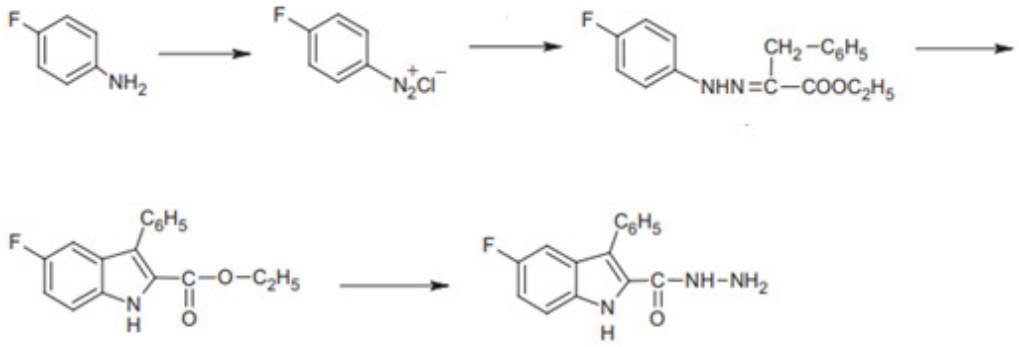


Figure 2.5: Synthesis reaction of 5-fluoro-3-phenyl-1H-indole-2-carbohydrazide

Hasan, Thomas and Gapil (2011) reported that 4-nitrobenzoic acid hydrazide can be prepared by the reaction between methyl-4-nitrobenzoate ester and hydrazine hydrate. Before the formation of the hydrazide, methyl-4-nitrobenzoate ester was synthesized from the reaction of 4-nitrobenzoic acid and absolute methyl alcohol in the presence of concentrated sulphuric acid. The reaction was heated under reflux for 4 hours. The reaction mixture was extracted with water, dichloromethane followed by sodium bicarbonate solution after completion of reaction. The crude

ester formed was washed, filtered and recrystallized to obtain the pure methyl-4-nitrobenzoate. Then, the synthesized ester (7g, 0.041 mol) and hydrazine hydrate (80%, 13 mL) were dissolved in absolute ethanol (40 mL) and reaction mixture was refluxed for 8 hours. After completion of reaction, the excess hydrazine was distilled off while the crude solid was collected, washed with water recrystallized from 30% aqueous ethanol to obtain the pure hydrazide. Figure 2.6 shows the reaction for synthesis of 4-nitrobenzoic acid hydrazide.

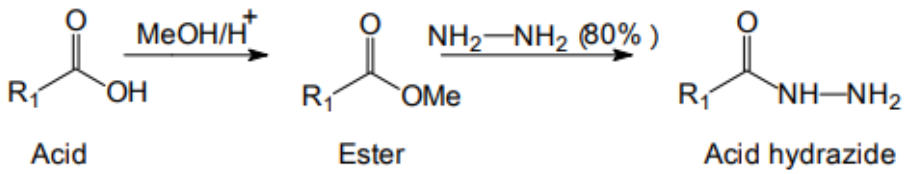


Figure 2.6: Synthesis of 4-nitrobenzoic acid hydrazide

2.2 Synthesis of 1,3,4-oxadiazoles

Bala et al. (2014) had stated that 1-(4-methoxyphenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)propan-1-one was synthesized from the reaction of β-benzoyl propionic acid and aryl hydrazide. Before that, the aryl hydrazide was synthesized from the aromatic ester, which was produced from the substituted aromatic acids through Fischer esterification, and hydrazine hydrate in presence of ethanol. Next, equimolar (1 M) of aryl hydrazide and β-benzoyl propionic acid was dissolved in 5 mL of phosphorous oxychloride. The reaction mixture was heated under for 6 to

7 hours then cooled to room temperature after completion of reaction and poured onto the ice. The reaction mixture was neutralized with sodium bicarbonate solution. Filtration, washing with water and recrystallization from methanol were done to yield the product. Figure 2.7 shows the scheme reaction for the synthesis of (4-methoxyphenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)propan-1-one.

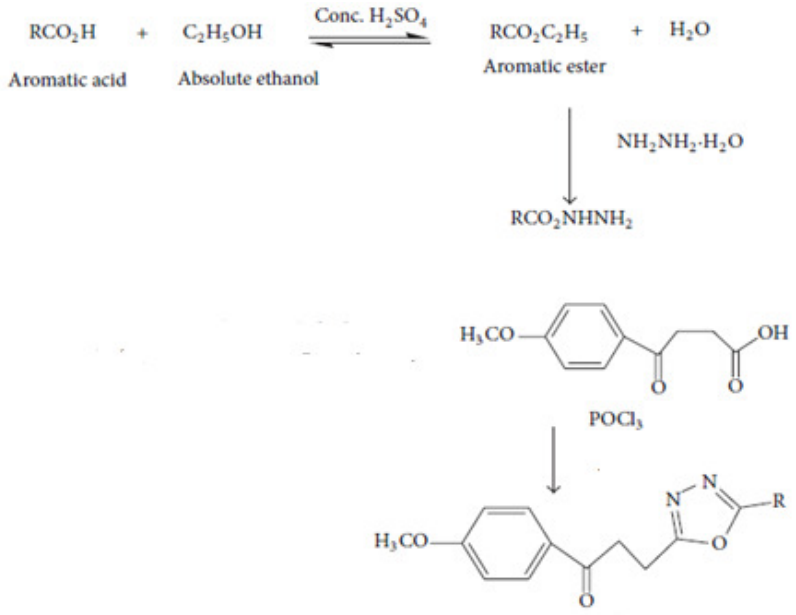


Figure 2.7: Synthesis of (4-methoxyphenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)propan-1-one

Based on Modi and Modi (2012), different types of methods were suggested to synthesis the 1,3,4-oxadiazoles. Firstly, 5-(4-nitro) phenyl-3H-1,3,4-oxadiazoline-2-thione was being synthesized by using the conventional method. Hydrazide (28 mmol, 5.13 g) that produced from the condensation of ethyl 4-nitrobenzoate and hydrazine hydrate was being reacted with the potassium hydroxide solution in the solvent of ethanol. Then, carbon disulfide (35 mmol) was added to the reaction

mixture. The reaction mixture was concentrated under vacuum and the residue was transferred into ice and concentrated hydrochloric acid. The precipitate formed was then filtered off and recrystallized from the ethanol : water with the ratio of 4:1 to yield the thione. Figure 2.8 shows the synthesis of 5-(4-nitro) phenyl-3H-1,3,4-oxadiazoline-2-thione.

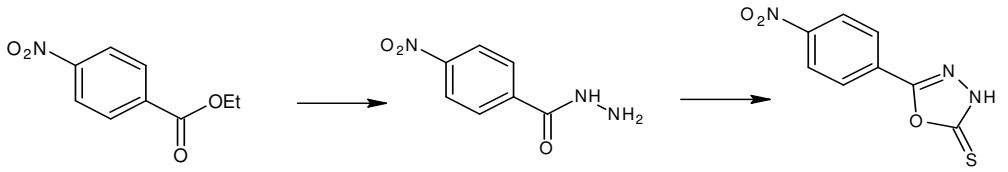


Figure 2.8: Synthesis of 5-(4-nitro) phenyl-3H-1,3,4-oxadiazoline-2-thione

Secondly, Modi and Modi (2012) also suggested that synthesis of 5-(4-nitro) phenyl-2-n-tetradecylthio-1,3,4-oxadiazole by using microwave method. It was being synthesized from the reaction between equimolar (0.036 mol) of 5-(4-nitro) phenyl-3H-1,3,4-oxadiazoline-2-thione with yriethylamine and 1-bromo tetradecane in absolute ethyl alcohol. The reaction mixture was irradiated in microwave for 55 seconds at 760 Watt. After that, the excess solvent was concentrated under vacuum while the residue was discharged into water. The precipitate that formed was then filtered and recrystallized from ethanol : water with the ratio of 1:1 to yield the product. Figure 2.9 shows the reaction for the synthesis of 5-(4-nitro) phenyl-2-n-tetradecylthio-1,3,4-oxadiazole.

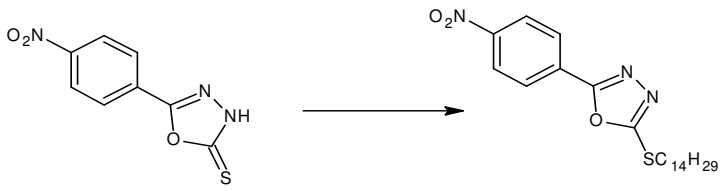


Figure 2.9: Synthesis of 5-(4-nitro) phenyl-2-n-tetradecylthio-1,3,4-oxadiazole

The reflux of a mixture of thiophene-2-carbohydrazide (1 g, 0.0078 mol) and benzoic acid derivatives (1 g, 0.008 mol) with the presence of phosphoryl chloride (0.078 mmol, 7.3 mL) yield the 1,3,4-oxadiazole products. The reaction mixture was under refluxed at 100 ℃ for 3 to 4 hours. Excess use of phosphorous oxychloride was perhaps to act as solvent in the reaction. The reaction was found to continue without any additional organic solvent thus the environmental pollution was being decreased (Kolli, 2016). Figure 2.10 shows the synthesis of 1,3,4-oxadiazole from thiophene-2-carboxylic acid.

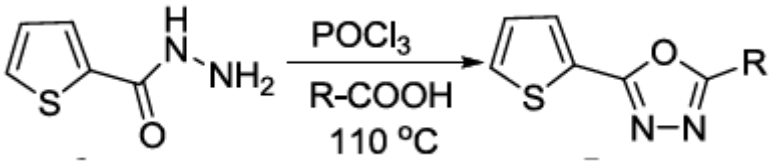


Figure 2.10: Synthesis of 1,3,4-oxadiazole from thiophene-2-carboxylic acid

Salahuddin et al. (2017) had reported that 2,5-disubstituted-1,3,4-oxadiazoles derivatives can be synthesized by the reaction between the substituted aromatic hydrazides with either aromatic acid derivatives in the presence of phosphorous oxychloride or in carbon disulfide in the presence of potassium hydroxide solution which shown in the Figure 2.11.

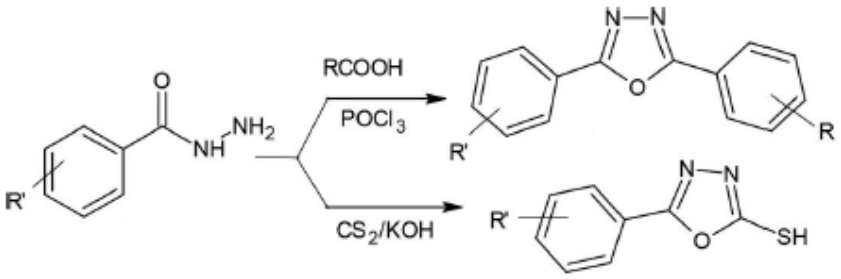
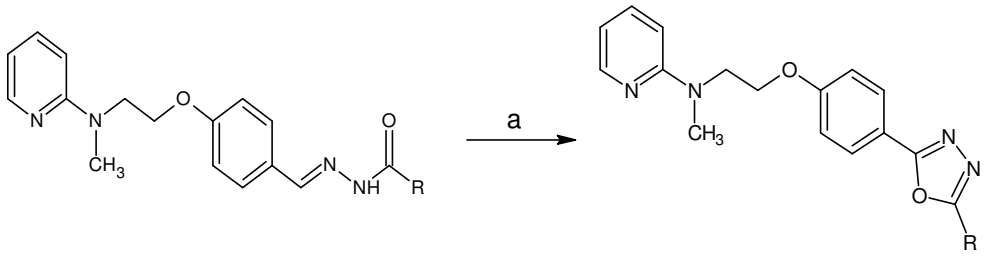


Figure 2.11: Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles derivatives

Salahuddin et al. (2017) also reported that cyclization of *N*-acylhydrazones with chloramine-T can synthesize the 5-substituted 1,3,4-oxadiazoles under microwave irradiation. The reaction mixture was irradiated for 20 to 50 minutes at 80 to100 ℃. Figure 2.12 shows the reaction for synthesis of 5-substituted 1,3,4-oxadiazoles.



a: Chloramine-T, MW, 20-50 min, 80 to100 ℃

Figure 2.12: Synthesis of 5-substituted 1,3,4-oxadiazoles

According to Jayaroopa, Ajay and Vasanth (2013), 2,5-disubstituted-1,3,4-oxadiazoles was synthesized from the reaction of equimolar (0.01 mol) of stearic acid hydrazide and suitable aliphatic or aromatic acids in the presence of phosphorous oxychloride while the steric acid hydrazide was synthesized under

reflux for 3 hours at 100 ℃ from the reaction between ethyl oleate and hydrazine hydrate in absolute ethanol. The reaction mixture was refluxed on water bath at 100 ℃ for 4 to 5 hours. Then, the reaction mixture was cooled to room temperature before poured into the ice. Sodium bicarbonate solution was added to neutralize the mixture. The resulting solid product was filtered, dried and recrystallized from 80 % ethyl alcohol to yield the product. Figure 2.13 shows the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles.

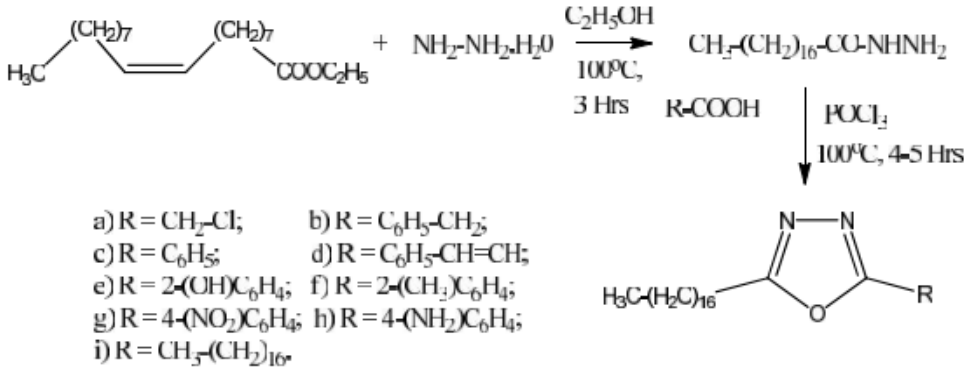


Figure 2.13: Synthesis of 2,5-disubstituted-1,3,4-oxadiazole

2.3 Antioxidant activity

There are two major evaluations to measure and analyze the antioxidant activity of compounds which are the in vivo and in vitro methods. Generally, in vivo antioxidant evaluations, the sample compounds that measured are normally administered to the testing animals at a known dosage regimen. In vitro antioxidant

evaluations are normally study about the free radical scavenging that is more straightforward and easy to perform. Hence, it is critical for the researchers to clarify the suitable method for analysis the antioxidant activity to avoid wastage of time (Alam, Bristi and Rafiquzzaman, 2013).

Table 2.1: Examples of in vivo and in vitro methods.

In vivo methods	In vitro methods
Ferric reducing ability of plasma	DPPH scavenging activity
Reduced glutathione (GSH) estimation	Hydrogen peroxide scavenging (H2O2) assay
Glutathione peroxidase (GSHPx) estimation	Nitric oxide scavenging activity
Superoxide dismutase (SOD) method	Peroxynitrite radical scavenging activity

2.3.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH assay is a method that developed by Blois to determine the antioxidant activity by using a stable a stable free radical α, α-diphenyl-β-picrylhydrazyl. This method has been widely used due to it is a rapid and easy to measure the ability of compounds to acts as free radical scavengers and evaluate antioxidant activity (Kedare and Singh, 2011). DPPH is characterized as a stable free radical because of the delocalization of the extra electron over the molecule to prevent it from

dimerize. The solution of DPPH is in deep violet color and characterized by an absorption band in ethanol solution at 517 nm due to the presence of unpaired electron on one nitrogen atom. The deep violet color of DPPH solution becomes yellow solution when it is mixed with the substrate which can donate hydrogen atom as the DPPH is in the reduced form (Alam, Bristi and Rafiquzzaman, 2013).

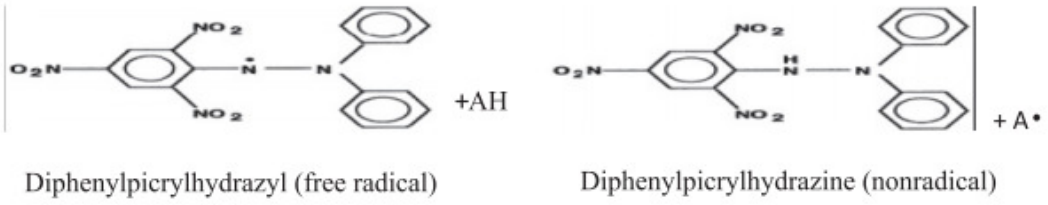


Figure 2.14 Reduction of DPPH free radical to DPPH-H non-radical

The change in optical density of DPPH radicals is monitored to evaluate the antioxidant potential of the compounds. The color changes from deep violet to yellow solution represented that the free radical DPPH is reduced where the absorption is at 517 nm. Parameter that often used to interpret the antioxidant activity of compounds is the inhibitory concentration, IC_{50} which well-defined as the concentration of antioxidant where the free radical activity is inhibited by 50 %. The percentage of free radical DPPH inhibition can be calculated from the equation below (Hangun-Balkir and McKenney, 2012).

$$\text{Inhibition } (\%) = [(A_{blank} - A_{sample})/A_{blank}] \text{ X } 100\ \%$$

Where A_{blank} is the absorbance of blank and A_{sample} is the absorbance of sample.

CHAPTER 3

MATERIALS AND METHODS

3.3.4 Purification of products through recrystallization

Crude products of carboxylic hydrazide and 1,3,4-oxadiazole obtained after refluxed were purified by recrystallization using hot ethanol. The ethanol was boiled and added into the beaker containing products to dissolve it. The hot mixture was filtered through cotton wool in glass funnel on a hot plate to eliminate the insoluble substances present. Formation of crystals on the glass funnel or wall of beaker was avoided as it was a quick filtration process. A few boiling chips were added to the filtrate and allowed to boil until saturated. Then it was left to evaporated and dry until the product was reformed. The reformed product was rinsed with cold ethanol a few times and the solution was sucked out to obtain the pure product. It was then dried in the oven, collected into specimen tube and weighed. Thin Layer Chromatography (TLC) was used to verify the purity of the compound followed by characterization using Fourier Transform Infrared Spectrophotometer (FTIR), and Nuclear Magnetic Resonance (NMR).

3.4 Characterization of products

3.4.1 Thin Layer Chromatography (TLC)

Chromatography is a method used to separate, identify and purify of the individual compounds of a mixture for quantitative and qualitative analysis. The principle of this method is where the mixture solution is applied onto the stationary phase and separated by the mobile phase. Thin Layer Chromatography (TLC) is often used due to ease and simplicity. TLC was performed on a sheet of aluminium foil that coated with adsorbent material which is the silica gel. The silica gel is act as the stationary phase while the mixture of solvent acts as mobile phase. The TLC plate was placed in a chamber for elution after the samples were dotted on the baseline by using capillary tube. The rate of travelling of the compounds on the TLC plate was depend on their attraction toward the stationary phase due to their different in polarity. The TLC plate was exposed under ultraviolet light to observe and mark the spots when the mobile phase reached the solvent front. The spots shown can be differentiated by calculate their retention factor by using the formula shown below.

$$R_f = \frac{\text{distance travelled by component from baseline (cm)}}{\text{distance between solvent front and baseline (cm)}}$$

The solvent system used for carboxyl hydrazide and 1,3,4-oxadiazole derivatives is ethyl acetate and hexane with the ratio of 1:1.

3.4.2 Fourier Transform Infrared Spectrophotometry (FTIR)

Infrared spectrophotometer shows the wavelength and the intensity of absorption of a sample based on different functional groups that presence in the sample that provide the information of the structure. Peaks that shown in the spectrum are represent different functional groups in the compound where the common functional groups can be determined from a standard table of characteristic of IR absorptions. The analysis performs at the frequency of 4000 cm^{-1} to 400 cm^{-1} by preparing the synthesized solid compounds in KBr pellets. In this project, IR spectra were used to determine the major functional groups present in the synthesized compounds.

3.4.3 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance spectrometer is used to define the purity and structure of compounds. It provides the magnetic properties of distinct atom nuclei that depend on the nucleus processing spin. The atom nuclei absorb the external radiation when it was exposed to the magnetic field. The strength of magnetic field increases because of the resonance frequency, peak intensity and absorption energy increases. To elucidate the structure of compounds, NMR analyses such as ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC were performed.

About 10 mg of compound was placed into a sample vial and dissolve the compound by using deuterated chloroform and dimethyl sulfoxide solvent. The dissolved compound is then subjected to the NMR tube until a height of 4 cm and capped to avoid evaporation of solvent. Purpose of using deuterated chloroform in NMR analysis is that it will not exchange its deuterium with protons of the compounds and thus the analysis of the compounds will not be interfered.

3.4.4 Melting point apparatus

Melting point apparatus is used to determine the melting point of each of the synthesized compounds. Adequate amount of sample was loaded into a capillary tube before it was placed into an apparatus. To ensure fast determination of melting point, the melting temperature was set in higher value. The sample was observed through the magnifying lens of the apparatus while the temperature of the apparatus will increase by itself. The temperature was recorded in a range from where it started to melt till it completely melted. The temperature will be altered if there is presence of impurities in the compounds. The apparatus is put aside for cooling down after done for a measurement.

3.5 Antioxidant Activity using DPPH assay

Antioxidant activity of carboxyl hydrazide and 1,3,4-oxadiazoles were carried out by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay while standard antioxidant butylated hydroxytoluene (BHT) was used as positive control. As the violet color of DPPH turns yellow, it means that the antioxidant activity is increase as the absorbance decreases. The whole experiment is carried out in the dark places as it was light sensitive.

3.5.1 Preparation of DPPH solution

0.00348 g of DPPH was dissolved in methanol and diluted to volume with methanol in a 100 mL volumetric flask which wrapped with aluminium foil. The solution is shaken vigorously and incubated in a dark room.

3.5.2 Preparation of DPPH free radical assay

To prepare 500 ppm solution, 5 mg of each compound was dissolved in methanol and diluted to volume in a 10 mL volumetric flask. A series of dilution of the sample stock solution was done at 200, 100, 50, 25, 12.5 and 6.25 ppm. 1 mL of these diluted solutions was mixed with 4 mL of DPPH solution in sample bottles wrapped with aluminium foil. A blank sample was prepared by adding 4 mL of DPPH solution with 1 mL of methanol in a sample bottle wrapped with aluminium foil. All the samples were shaken vigorously and incubated in dark at room temperature for 30 minutes. The absorbance value for all the solution in the sample bottles was

measured at 517 nm using UV-Vis spectrophotometer with methanol as blank. All samples and readings were prepared and measured in triplicate. The percentage of radical scavenging was calculated by using the following equation:

% Radical scavenging = $[(A_{blank} - A_{sample})/A_{blank}]$ X 100 %

Where,

A_{blank} is the absorbance of blank after 30 minutes and

A_{sample} is the absorbance of sample after 30 minutes.

3.6 Calculations

I. Mass (g) = number of mole (mol) x molecular weight (g/mol)

Used to calculate mass of starting materials required for syntheses

II. Volume (mL) = mass (g) x density (g/mL)

Used to convert mass of starting materials into amount of volume needed for syntheses

III. Percentage yield (%) = $\frac{\text{Experimental mass of product (g)}}{\text{Theorectical mass of product (g)}}$ x 100%

Used to calculate percentage yield of product obtained from syntheses

CHAPTER 5

CONCLUSION

5.1 Conclusions

In this project, indole ester, carboxyl hydrazide and four new 1,3,4-oxadiazoles such as **JL1**, **JL2**, **JL3** and **JL4** were successfully synthesized. Structure and purity of carboxyl hydrazide and 1,3,4-oxadiazoles such as **JL1**, **JL2**, **JL3** and **JL4** were characterized by using ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, FT-IR, TLC and melting point apparatus. These compounds were also evaluated for their antioxidant activity by using DPPH assay. The results of antioxidant activity indicated that the synthesized compounds show weak antioxidant activity below the concentration of 200 ppm.

5.2 Future Perspectives

Carboxyl hydrazide derivatives can be synthesized by other starting materials compound. Various types of carboxyl hydrazide derivatives can be synthesized to give other types of biological and pharmaceutical activities. Besides, synthesis of carboxyl hydrazide derivatives can be carried out by microwave radiation or by ionic liquid as solvent. On the other hand, synthesis of 1,3,4-oxadiazole can be done by reacting other kinds of benzoic acid derivatives with other hydrazide derivatives. Biological and pharmaceutical activities such as antibacterial, antifungal, anticancer activities can be tested in the future.

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