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

By

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ABSTRACT

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

NG YU XUAN

1,3,4-oxadiazoles are important in various fields and have been involved in many studies by researchers. 1,3,4-oxadiazoles have many biological activities, for example, antifungal, antibacterial and anti-oxidant activities. A carboxylic acid hydrazide was synthesized from the reaction between an indole ester and a hydrazine hydrate and used as a starting material of 1,3,4-oxadiazole derivatives. There were four 1,3,4-oxadiazoles being synthesized in this project. The 1,3,4-oxadiazole derivatives were synthesized from carboxylic acid hydrazide reacted with different benzoic acid derivatives with the presence of $POCl_3$. The percentage yields for the synthesized 1,3,4-oxadizoles were between 8% to 70%. For the characterization of the carboxylic acid hydrazide and the 1,3,4-oxadiazoles were using various instruments such as melting point apparatus, FT-IR, ^{1}H NMR,^{13}C NMR , DEPT, HMQC, and HMBC.

ABSTRAK

SINTESIS DAN KARAKTERISASI 1,3,4-OXADIAZOLES DENGAN INDOLE RING

NG YU XUAN

1,3,4-oxadiazoles penting dalam pelbagai bidang dan telah terlibat dalam banyak kajian oleh penyelidik. 1,3,4-oxadiazoles mempunyai banyak aktiviti biologi, misalnya aktiviti antijamur, antibakteria dan anti-oksidan. Hidrazida asid karboksilik disintesis dari tindak balas antara ester indol dan hidrat hidrazin dan digunakan sebagai bahan permulaan turunan 1,3,4-oksadiazol. Terdapat empat 1,3,4-oksadiazol yang disintesis dalam projek ini. Derivatif 1,3,4-oksadiazol disintesis dari hidrazida asid karboksilik yang bertindak balas dengan turunan asid benzoat yang berbeza dengan adanya $POCl_3$. Hasil peratusan untuk 1,3,4-oksadizol yang disintesis adalah antara 8% hingga 70%. Untuk pencirian hidrazida asid karboksilik dan 1,3,4-oksadiazol menggunakan pelbagai instrumen seperti alat lebur, FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, dan HMBC.

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Lastly, I would like to thank my family members for their moral and physical support throughout my three years in Universiti Tunku Abdul Rahman.

DECLARATION

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

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NG YU XUAN

APPROVAL SHEET

This project entitled "**SYNTHESIS AND CHARACTERIZATION OF 1,3,4-OXADIAZOLES BEARING AN INDOLE RING**" was prepared by **NG YU XUAN** and submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Honours) in Chemistry at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Dr. Sim Kooi Mow) Date: …….

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Department of Chemical Science

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FACULTY OF SCIENCE

UNIVERISITI TUNKU ABDUL RAHMAN

Date:_____

PERMISSION SHEET

It is hereby certified that **NG YU XUAN** (ID No: **16ADB02896**) has completed this final year project entitled "**SYNTHESIS AND CHARACTERIZATION OF 1,3,4-OXADIAZOLES BERAING AN INDOLE RING**" under the supervision of Dr. Sim Kooi Mow from the Department of Chemical Science, Faculty of Science.

I hereby to give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(NG YU XUAN)

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LIST OF ABBREVIATIONS

%	Percentage
℃	Celsius
δ	Chemical shiff(ppm)
^{1}H	Proton
^{13}C	Carbon-13
DEPT	Distortionless Enchancement by polarization Transfer
DMSO	Dimethylsulfate
EA	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier Transform Infrared
G	Gram
H_2O	Water
HCl	Hydrochloric acid
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Herz

mg	Milligram
mL	Milliliter
mmol	Millimole
Mol	Mole
NMR	Nuclear Magnetic Resonance
$POCl_3$	Phosphorous oxychloride
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 Indole

Indole is a heterocyclic compound made up of two cyclic compounds. The two compounds are benzene and a pyrrole ring which the pyrrole nucleus located at 2, 3 positions in the ring. Indole is a non-basic nitrogenous compound and it has molecular formula is C_8H_7N. The indole was named by combining indigo and oleum. Indigo is a natural blue dye that was popular in ancient civilizations. Indigo can modify to form isatin and oxindole. Adolf von Baeyer investigated indole by reducing the oxindole using zinc dust in 1866. The chemical structure of indole (Figure 1.1) was proposed by Adolf von Baeyer in 1869.

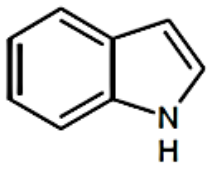
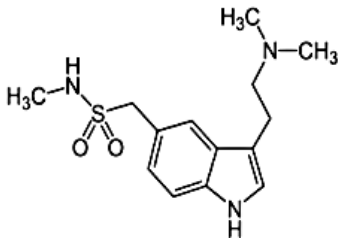
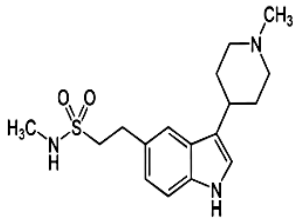
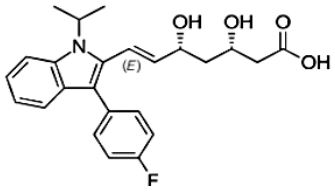
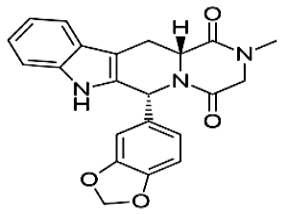


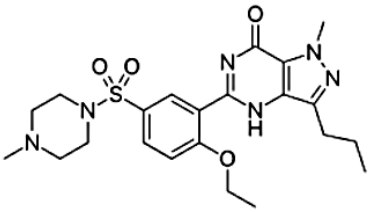
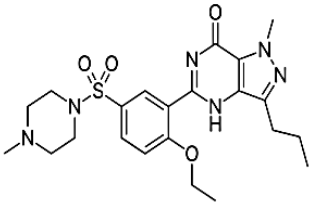
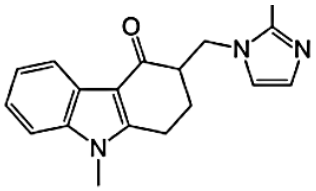
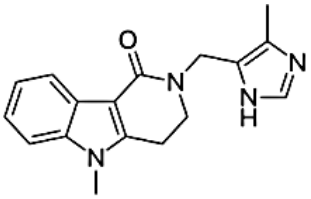
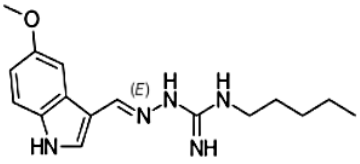
Figure 1.1: Structure of indole

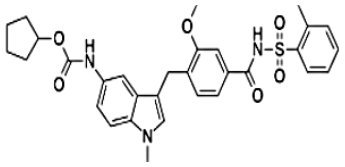
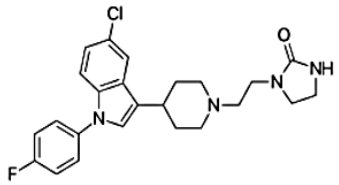
The indole derivatives can be found in natural products form in animals, plants, and microbial. Serotonin is considered as an indole derivative converted from tryptophan. Serotonin act as a neurotransmitter and widely involved brain activity (Hamid et al., 2017). Dragmacidin is extracted from marine natural product and it has properties of cytotoxicity (Gul and Hamann, 2005). Ajmaline and Reserpine are used to treat high blood pressure and mental disorder.

Indole derivatives have many biological activities including antimicrobial activity, anti-inflammatory, anticonvulsant, anticancer, antipsychotics, and antiviral activity. These biological activities make the indole derivatives play an important role in the pharmaceutical field.

Table 1.1: Indole drugs in Pharmaceutical field

Name	Chemical structure	Biological activities or treatment
Sumatriptan		Antimigraine
Naratriptan		Antimigraine
Fluvastin		Treatment of Hypercholesterolemia & lipoproteinemia
Tadalafil		Used to treat erectile dysfunction or pulmonary arterial hypertension

Sildenafil		used to treat erectile dysfunction and pulmonary arterial hypertension
Vardenafil		used for treating erectile dysfunction
Ondansetron		Antiemetic caused by cancer chemotherapy, radiation therapy, or surgery
Alosetron		Antiemetic
Tegaserod		To treat chronic idiopathic constipation

Zafirlukast		used for the chronic treatment of asthma
Sertindole		Antipsychotics

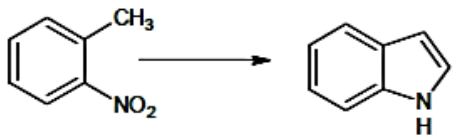
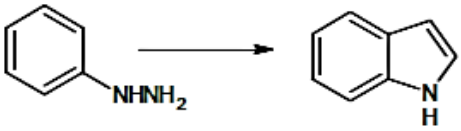
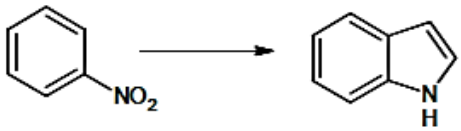
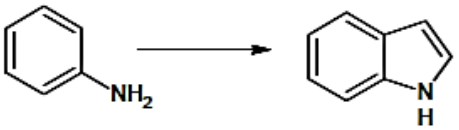
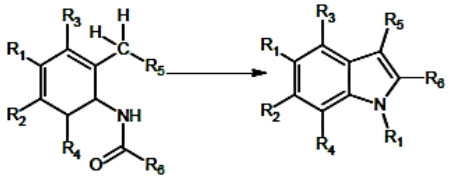
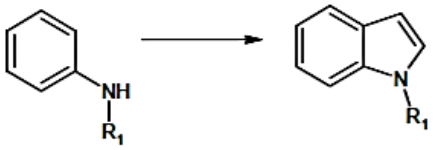
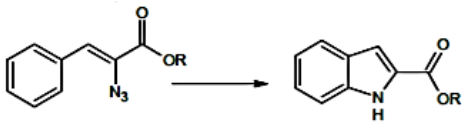
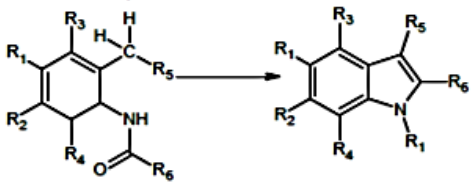
(Buvana et al., 2018)

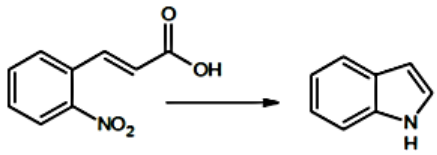
1.1.1 Fischer Indole Synthesis

There are many methods to synthesis indole derivatives. According to Kerzarea and Khedekar (2016) the methods for indole synthesis are Leimgruber-Batcho indole synthesis, Fischer indole synthesis, Bartoli indole synthesis, Bischler-Mohlau indole synthesis, Fukuyama indole synthesis, Gassman indole synthesis, Hemetsberger indole synthesis, Larock indole synthesis, Madelung indole synthesis and Baeyer-Emmerling indole synthesis. Table 1.2 shown that the methods for synthesis indole derivatives.

Table 1. 2: The methods of indole synthesis.

Method	Reaction

Leimgruber-Batcho indole synthesis	
Fischer indole synthesis	
Bartoli indole synthesis	
Bischler-Mohlau indole synthesis	
Fukuyama indole synthesis	
Gassman indole synthesis	
Hemetsberger indole synthesis	
Larock indole synthesis	
Madelung indole synthesis	

Baeyer-Emmerling indole synthesis	

(Kerzare and Khedekar, 2016)

Fischer Indole synthesis is one of the method for indole synthesis. Fischer Indole reaction is the reaction between equimolar of arylhydrazone and aldehyde or ketone with acid as catalyst. Fischer Indole was developed by Fischer and Jourden in 1883. The mechanism was proposed by Robinson and Robinson in 1918 (Van.Order and Lindwal, 1942).

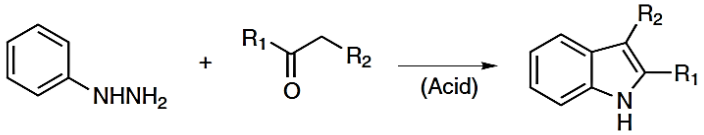


Figure 1.2: General Reaction Scheme of Fischer Indole Reaction (Wang, 2010).

The mechanism of Fischer Indole start with the hydrazone undergo tautomerization to form unsaturated hydrazine with the aid of acid.

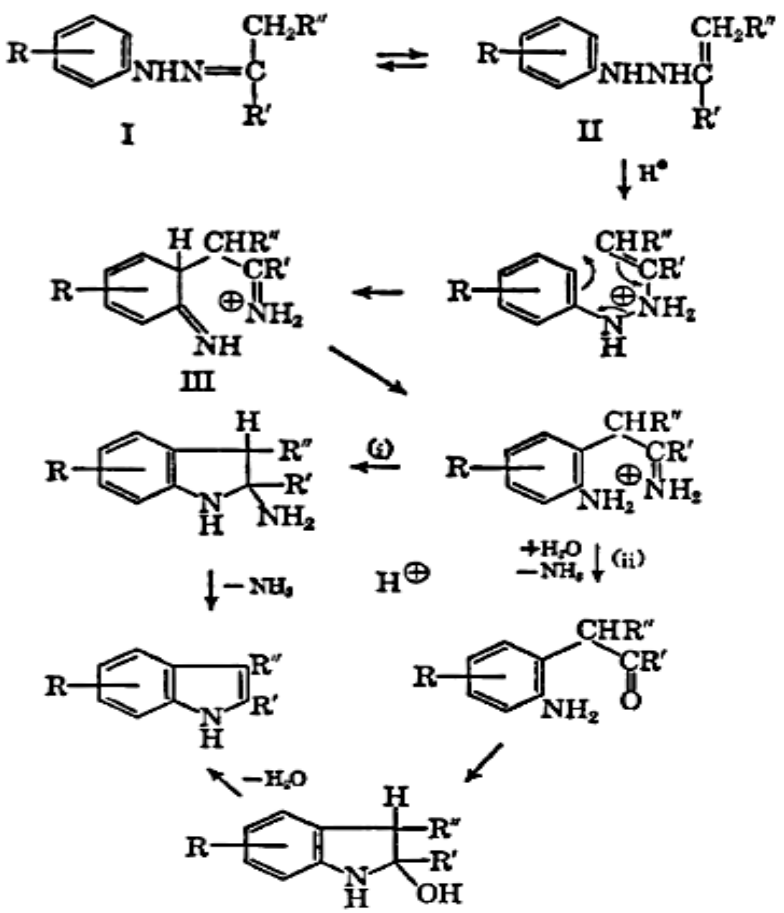


Figure 1.3: Robinson's mechanism (Robinson, 1963)

1.2 Hydrazine and Hydrazide

Hydrazine is a highly polar, colourless and flammable liquid in room temperature. Hydrazine has an ammonia like-odor. Hydrazine is known as a hazardous chemical. Hydrazine can absorb by human through inhalation, ingestion and through dermal. The symptoms of exposure to hydrazine can be classified into short-term exposure and long-term exposure. For short-term exposure symptoms, people normally will feel dizziness, nausea, irritation, and skin burns. Other than that, irritation of eyes,

nose and throat are one of the symptoms of short-term exposure to hydrazine. Long-term exposure to hydrazine will cause vomiting and itching of eyes. Hydrazine is known as a potential carcinogen (CDC - Immediately Dangerous to Life or Health Concentrations (IDLH): Hydrazine - NIOSH Publications and Products, 2020). Hydrazine also can cause central nervous depression, and damage internal organs such as kidneys and central nervous system.

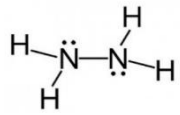


Figure 1.4: Hydrazine structure

Hydrazine is used as a chemical blowing agents to produce rigid or flexible polymer through thermal decomposition and polymer formulation (Mason and Sinha, 2014). Many hydrazine derivatives are useful in agriculture. Maleic hydrazide is used as plant growth regulator of potatoes and onions. It can inhibit the growth of sucker of tobacco. Hydrazine derivatives also apply in water treatment for removing corrosion. Hydrazine has the ability to scavenge oxygen in cooling system of the engines. Hydrazine fuels are mainly for bipropellant.

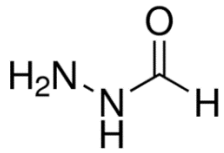


Figure 1.5: Hydrazide structure

In pharmaceutical field, hydrazine is the precursor of developing new drugs. Cefazolin (thiadiazole tetrazole derivative) has the properties of antibacterial. Carbidopa (alkylhydrazine) is use in treatment Parkinson disease. The other examples of hydrazine derivatives are Apresoline (hydrazine phthalazine) is used as reduce blood pressure and Furacin (nitrofurfuraldehyde semicarbasone) is used to treat coccidiocis of poultry (Troyan, 1953).

Table 1.3: Properties of hydrazine drivatives

Hydrazine derivative	Properties
1) Hydralazine	As a vasodilator drug in high blood pressure treatment. It has the property of restrain DNA methyltransferrase 1 (it inhibits methyl group transfer in cancer cell). It can induce hypoxia-induced factor (HIFα) which important in cancer chemotherapy. Hydrazine can cause DNA damage which induce lung tumor in mice.

2) Isoniazid	It is an anti-tuberculosis drug. But it is hepatotoxicity (can cause liver cancer).
3) Iproniazid	Iproniazid has the property to inhibit monoamine oxidase and used as antidepressant. It is forbid to use as antidepressant because of its hepatotoxicity.
4) Procarbazine	It is an anticancer drug. It is using to treat Hodgkin's lymphoma, malignant melanoma and brain tumors in adolescent. It is mutagenic to mammalian and bacterial.

(Mason and Sinha, 2014)

1.3 Oxadiazoles

Oxadiazole is a 5-membered heterocyclic ring which consists of two carbons, two nitrogens and one oxygen atom. There are four types of isomers of oxadiazole are 1,2,3-oxadiazoles, 1,2,4-oxadiazoles, 1,2,5-oxadiazoles and 1,3,4-oxadiazoles.

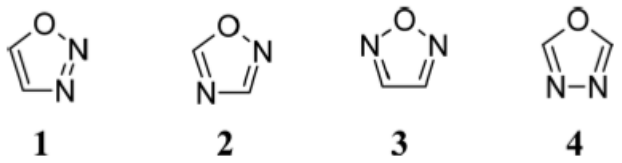


Figure 1.6: Isomers of oxadiazoles (Patel et al., 2014)

The oxadiazoles was found by Ticmann and Krugar in 1884. The oxadiazoles was named as furo[*ab*] diazoles (Patel et al., 2014). Oxadiazole is a weak base and has an inductive effect. The nitrogen of oxadizoles reduce the aromaticity of the ring and lead the oxadizoles to have conjugated diene character (Sanchit and Pandeya, 2011) .Carbon atoms of the oxadiazoles rarely occur reaction with electrophilic substitutions. This is because of the carbon is less electron density. For the electron substitutions occur in nitrogen, the electron-releasing group have to present in the oxadiazole ring. The oxadiazole moiety which carry the nitrogen atoms show various biological activities such as antibacterial, antimalarial, anti-inflammatory, antifungal, anticancer, antiretroviral, and as drugs for cardiovascular, central nervous system disorder and metabolicillnesses (Pitasse-Santos et al., 2018). Besides pharmaceutical field, oxadiazole moieties also show properties in luminescent applications,

herbicides, electron-transport materials, polymer and corrosion inhibitors (Pitasse-Santos et al., 2018).

1.4 1,3,4-oxadiazoles

Ainsworth synthesized 1,3,4-oxadiazoles in 1965 through thermolysis of ethylformate, formally hydrazine at atmospheric pressure. 1,3,4-Oxadiazole was known as oxybiazole, diazole, furro[*bb*'] diazole and biozole. The IUPAC name as 1,3,4-oxadiazole was used to replace the common names. The derivatives of 1,3,4-oxadiazole with amine and alkyl chains are important as electroluminescent materials (Mochizuki et al., 2000).

The traditional methods to synthesis 1,3,4-oxadiazole are dehydrative cyclization, electrophilic substitution, copper-mediated coupling reaction of aryl halides, oxidative cyclization with the presence of catalyst or oxidizing agents and Huisgen 1,3,4-oxadiazole synthesis (Wang et al., 2015).

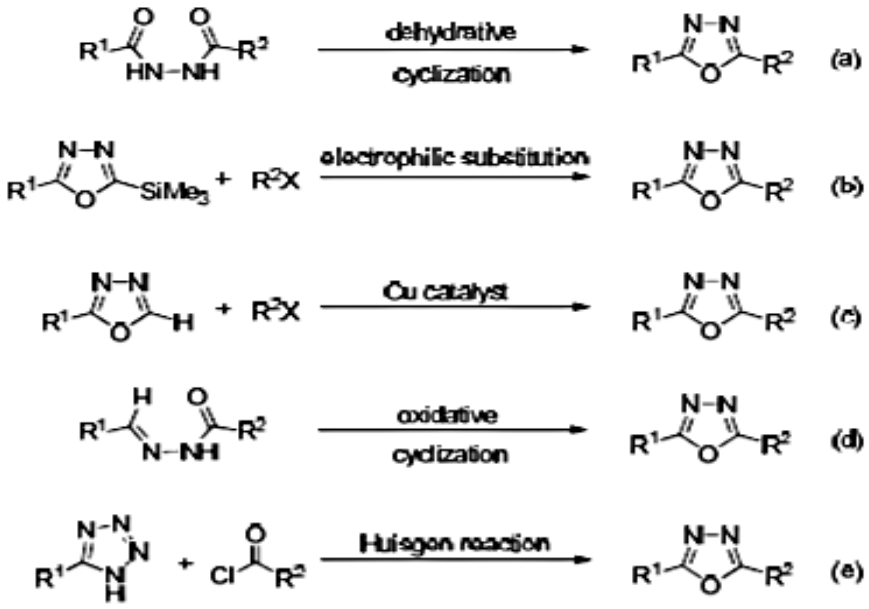


Figure 1.7: Synthesis methods of 1,3,4-oxadiazoles (Wang et al., 2015)

1.5 Objectives

I. To synthesis a carboxylic acid hydrazide and a series of 1,3,4-oxadiazoles bearing an indole ring.

II. To characterize the structure of the carboxylic acid hydrazide and 1,3,4-oxadizoles by using FTIR, ^{1}NMR, ^{13}NMR, DEPT, HMQC and HMBC.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of Hydrazide

According to Yale (1952), hydrazine salt can be obtained from the reflux of dimethyl cinchomeronate and hydrazine hydrate in methanol. Firstly to produce the hydrazine hydrate, 48 g. (0.24 mole) of dimethyl cinchomeronate in 960mL of methanol was reacted with hydrazine by adding the 85% of hydrazine hydrate dropwise into it. The mixture was reflux for 6 hours. After 6 hours, the solution was cooled to room temperature and the solid was being filtered. The solid was recrystallized using ethanol as 95% solvent. The recrystallized solids was the hydrazine salts. Hydrazine salts was then dissolved in water and acidified using acetic acid. The solution will undergo separation then proceed to filtration. The filtrated solids will be recrystallized using dimethylformamide. Around 80% of percentage yield of hydrazine hydrate will be produced.

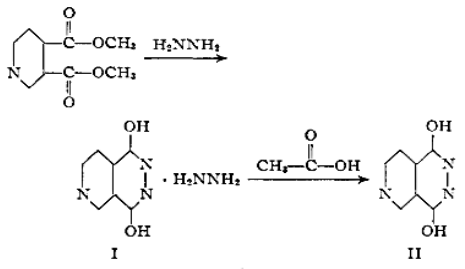


Figure 2.1: Reaction between dimethyl cinchomeronate and hydrazine hydrate in methanol (Yale et al., 1953)

Abdel-Aziz (2007) stated that 3-methylthiazolo[3,2-*a*]benzimidazole-2-carboxylic acid ethyl ester reacts with hydrazine hydrate enable the hydrazide to be synthesized. 2.6 g of 3-methylthiazolo[3,2-*a*]benzimidazole-2-carboxylic acid ethyl ester will react with 0.6 mL of 99% of hydrazine hydrate in 50 mL of absolute ethanol solution. The mixture was refluxed for 5 hours. After refluxing, the solution was being cooled to room temperature. Filtration was used to separate the white solid in the solution then the solid was being recrystallized. Recrystallization solution can be used are ethanol or dimethylformamide. The final product which is hydrazide will be white colour solid with 76% percentage yield.

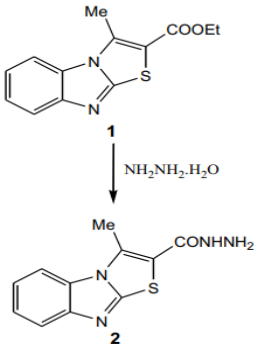


Figure 2.2: Reaction between 3-methylthiazolo[3,2-*a*]benzimidazole-2-carboxylic acid ethyl ester and hydrazine hydrate (Abdel-Aziz et al., 2007)

Abdel-Aziz and Abdel-Rahman (2010) reported that pyrazine-2-carboxylic acid hydrazide synthesized from esterification and hydrazinolysis of pyrazine-2-carboxylic acid. 0.124 g (1.0 mmol) of pyrazine-2-carboxylic acid in 30 mL of chloroform at 10℃. An equal mole of triethylamine was added to the stirred solution follow by an equal mole of ethyl chloroformate was added dropwise with 10 minutes of nitrogen flow. The mixture was allowed to stir for 30 minutes. Over 15 minute's period, 10 mmol of methanol was added and stirred the solution for 12 hours at room temperature. An oily crude product will be obtained by evaporating the solvent. The crude product will dissolve in 30 mL of methanol. 5 mmol of hydrazine monohydrate was added in the solution and then reflux for 6 hours. The solution was allowed to cool and undergo filtration to obtain the precipitates. The product was being recrystallized using ethanol as the solvent. A yellowish solid was produced with 80% yield.

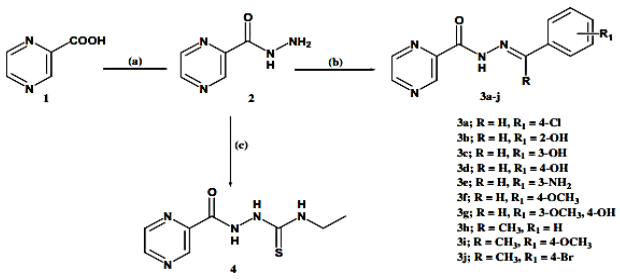


Figure 2.3 : Outline synthesis by using pyrazine-2-carboxylic acid hydrazide (Abdel-Aziz and Abdel-Rahman, 2010)

Cacic et al. (2006) stated that (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid ethyl ester undergo hydrazinolysis will produce (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid hydrazide. The reaction between (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid ethyl ester in 120 mL of methanol added with 12 mL of 100 % hydrazine hydrate. The mixture put at room temperature for 24 hours. The desired product undergo separation, vaccum filtration and recrystallization. During vaccum filtration, the product need to wash with methanol and light petroleum. The solvent that used in recrystallization is water or diluted acetic acid. The (7-hydroxy-2-oxo-2*H*-chromen-4-yl) acetic acid hydrazide will have 70 % yield.

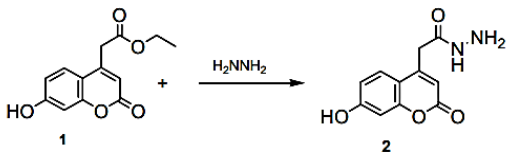


Figure 2.4 : Hydrazinolysis of (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid ethyl ester (Cacic et al., 2006)

According to Cihan-Üstündağ et al. (2016), ethyl 5-fluoro-3-phenyl-1*H*-indole-2-carboxylate react with 98 % of hydrazine hydrate can synthesis 5-fluoro-3-phenyl-1*H*-indole-2-carbohydrazide. Firstly, the 0.02 mol of ethyl 5-fluoro-3-phenyl-1*H*-indole-2-carboxylate in 20 mL of ethanol react with 98 % hydrazine hydrate in the reflux condition. The reflux duration is 6 hours. After refluxing, the mixture was cooled and being filtered. The brown solid will be obtained from the recrystallization using ethanol-chloroform.

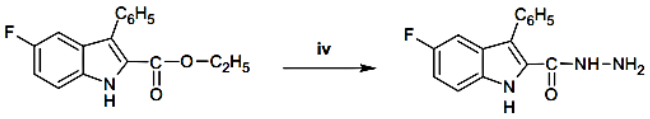


Figure 2.5: Synthesis of 5-fluoro-3-phenyl-1*H*-indole-2-carbohydrazide (Cihan-Üstündağ et al., 2016)

2.2 Synthesis of 1,3,4-Oxadiazoles

According to Frohlichova et al. (2009), The 1,3,4-oxadiazole derivatives were synthesized from acylthiosemicarbazides. 0.4 mmol of acylthiosemicarbazides in 4 mL of absolute ethanol solution react with 0.09 mmol of mercuric oxide. The mercuric oxide was distributed adding into the solution in 30 minutes period. The mixture have to reflux for 2 hours and then filter when it is warm. The black solid was washed with 3 mL of hot ethanol for 3 times. The solutions was concentrated into small volume and leave overnight at room temperature. The solution then will undergo filtration to get the solid. Drying and recrystallization were necessary for the purification of the product. This synthesis method can get 13 % yield of 1,3,4-oxadiazoles.

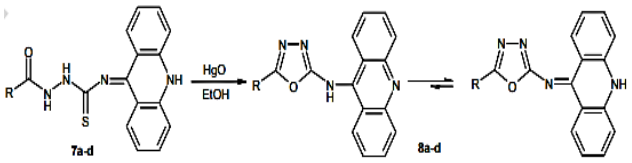


Figure 2.6 : 1,3,4-oxadizoles synthesis scheme(Frohlichova et al., 2009).

For the stirring 2-(2-chlorophenoxy)benzoic acid hydrazide solution, a 60 mL solution that made up from 25.8 mmol sodium bicarbonate and 25.9 mmol of dioxane in 40 mL of water were mixed together. The solution was allowed to stir at 25 ℃ for 5 minutes. After stirring for 5 minutes, about 26 mmol of cyanogen bromide was

added. The solution will add with 200 mL of water after 3 hours. The product formed will collect through filtration and recrystallize using ethanol. The oxadiazoles produced through this method is around 80% yield.

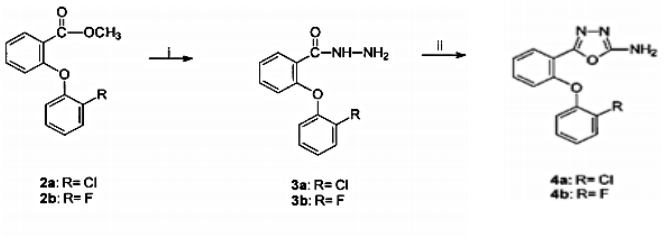


Figure 2.7 : Formation of 1,3,4-Oxadiazole (Rad et al., 2004).

Triloknadh et al. (2018) stated that reaction of 3-((2,4-dinitrophenyl)thio)propanoic acid and hydrazide acid with the aid of phosphorus oxychloride can produce 1,3,4-oxadiazole derivatives. Equimolar (0.36 mmol) of 3-((2,4-dinitrophenyl)thio)propanoic acid and hydrazide acid with 4 mL of phosphorus oxychloride will reflux for 5 to 6 hours at 90 ℃. After reaching the time of reflux, the solution was then poured into 50 mL of chilled water dropwised. The ammonia solution was used as neutralizing agent. The 1,3,4-oxadizoles produced can up to 75 % yield.

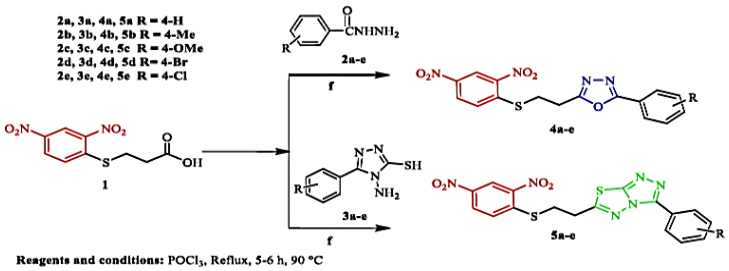


Figure 2.8 : Synthesis of 1,3,4-oxadiazoles and 1,3,4-thiodiazoles (Triloknadh et al., 2018)

For the synthesis of 1,3,4-oxadiazole tagged thieno[2,3-*d*] pyrimidine derivatives, Harikrishna et al. (2012) proposed the reaction of 4-(substituted phenylamino)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylic acids and carboxylic acid hydrazide. A mixture that made up from 0.01 mol of 4-(substituted phenylamino)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylic acids and carboxylic acid hydrazide and 20 mL phosphorus oxychloride was being refluxed for 5 hours. The mixture was cooled to room temperature after 5 hours. When it reached to room temperature, the mixture was poured into crushed ice and keep stirring it. The solution was need to leave for 24 hours then proceed to filtration to get the crude oxadiazole. During filtration, the sodium hydroxide was used to neutralize the acid and the product was washed with cold water. The crude 1,3,4-oxadiazole was allowed to dry. The dried 1,3,4-oxadiazole was proceed to recrystallization using chloroform and hexane. The purified 1,3,4-oxadiazole will be in around 80 % yield.

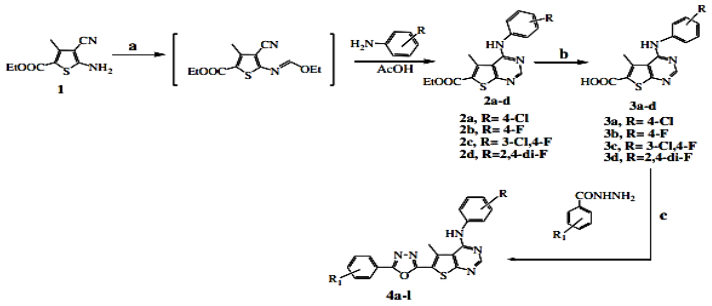


Figure 2.9 : Synthesis of 1,3,4-oxadiazole tagged thieno[2,3-*d*]pyrimidine derivatives (Kotaiah et al., 2012).

2-(phenoxymethyl)-1*H*-benzimidazole was synthesized from o-phenylenediamine and phenoxyacetic acid first. Then a hydrazide (2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide) will be produced by adding hydrazine hydrate with the anhydrous potassium carbonate dissolve in dry acetone. The present of $POCl_3$ will cause condensation with the temperature increase to 110-120 ℃. The final product will be 2-(phenoxymethyl)-1-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-1*H*-benzimidazole by using this synthesis method (Shaharyar et al., 2017).

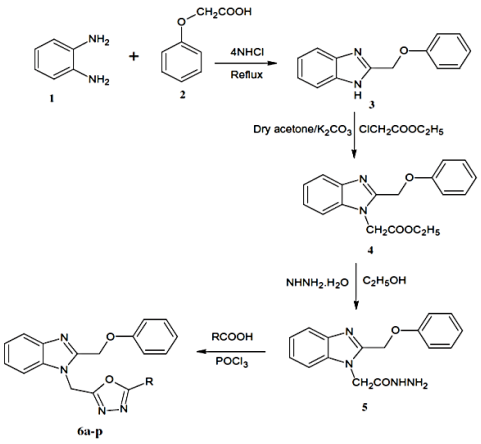


Figure 2.10 : Formation of 2-(phenoxymethyl)-1-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-1*H*- benzimidazole (Shaharyar et al., 2017).

Siddiqui (2013) reported 1,3,4-oxadiazole can be synthesized through cyclization via CS_2 of hydrazide. For synthesis 5-benzyl-1,3,4-oxadiazole-2-thiol, 0.3 moles of KOH was added to 0.1 moles of phenylacetic acid hydrazide in 25 mL ethanol was heated with stirring. When all KOH was dissolved, 0.1 moles of carbon disulfide was then introduced into the reaction. The mixture have to reflux for 4 hours. During refluxing, the H_2S(hydrogen sulfide) will be released. After 4 hours, the mixture was had been acidified to pH 3 by adding diluted HCl. The mixture was then being filtrate to get the yellow precipitate. The crude 1,3,4-oxadiazole was rinsed using distilled water and allow it to dry. Ethanol was used as the recrystallize solvent for the crude 1,3,4-oxadiazole. The purity of the purified 1,3,4-oxadiazole can be checked with TLC by using ethyl acetate and hexane as solvent. The ratio of ethyl acetate and hexane is 60:40. The pure product will give single spot under UV lamp as result. The 5-benzyl-1,3,4-oxadiazole-2-thiol can be get through this reaction is 87% yield.

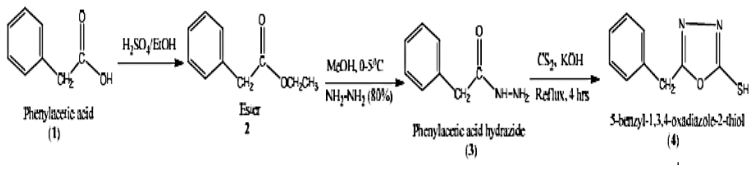


Figure 2.11 : Formation of 5-benzyl-1,3,4-oxadiazole-2-thiol(Siddiqui et al., 2013)

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals

Table 3.1: Chemicals that used to synthesis indole ester

CHEMICALS	MANUFACTURER	COUNTRY
Acetic acid	Merck	Cermany
Ethyl acetate	LAB-SCAN	Ireland
Absolute ethanol	Acros Organic	Belgium
Anhydrous sodium sulphate	Fischer Scientific	UK
Concentrated Hydrochloric acid	Fischer Scientific	UK

Table 3.2: Chemicals that used to synthesis carboxylic hydrazide

CHEMICALS	MANUFACTURER	COUNTRY
Hydrazide hydrate	Merck	Germany
Absolute ethanol	Acros Organics	Belgium

Table 3.3: Chemicals that used to synthesis 1,3,4-oxadiazole

CHEMICALS	MANUFACTURER	COUNTRY
Benzoic acid	Merck	Germany
4-chlorobenzoic acid	Merck	Germany
4-methylbenzoic acid	Merck	Germany
2,4-dichlorobenzoic acid	Sisco Research Laboratory	India
Phosphorus oxychloric	Merck	Germany

Table 3.4: Solvents used in TLC

CHEMICALS	MANUFACTURER	COUNTRY
Ethyl acetate	LAB-SCN	Ireland
n-hexane	Merck	Germany

Table 3.5: Solvents used in NMR

CHEMICALS	MANUFACTURER	COUNTRY
DMSO-d_6	Fischer Scientific	UK

3.2 Instruments

Types of Instrument	Model
FT-IR spectrophotometry	Perkin Elmer 2000-FTIR Spectrophotometer Spectrum RX I
NMR	FT-NMR Spectrometer JEOL JNM ECX-400
Melting point apparatus	Barnstead Electrothermal 9100

Table 3.6: Types of instrument used

3.3 Methodology

3.4 Characterization of carboxylic acid hydrazide and 1,3,4-oxadiazole derivatives

3.4.1 Thin Layer Chromatography

Thin Layer Chromatography is a plate that coated with silica gel and useful in separation of mixture. Thin Layer Chromatography enable to track the reaction progress, determine the type of compounds in the provided structure and check for the purity of the product (Bele and Khale, 2010). In order to check the reaction progress, the formation of new spot or disappear of the spot of reagent. The Thin Layer Chromatography can be classified into 3 steps: spot the sample, development of the sample movement on the plate, and visualize the spots using UV lamp. Firstly, the dissolved samples were spotted on the TLC plate using a tip of micropipette. Then it will be transfer to a chamber that contain solvents. The coated silica on the plate act as a stationary phase, the solvent act as the mobile phase. The sample will move along with the solvent on the plate. The R_f value were obtained from the formula below. The solvents used for 1,3,4-oxadiazole derivatives are ethyl acetate and hexane in 1:1 ratio.

$$R_f = \frac{Distance\ of\ compound\ traveled\ from\ baseline}{Distance\ of\ solvent\ traveled\ from\ baseline\ (mm)}$$

3.4.2 Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy can use to identify the functional groups present in the samples. A graph of % transmittance against frequency (cm^{-1}) will be shown after testing the sample. We can identify the functional groups by comparing the sample data with the theoretical values. The frequency that used to analysis is 4000 cm^{-1}- 400 cm^{-1}. The sample will be in a pellet form with KBr powder.

3.4.3 Nuclear Magnetic Resonance

Nuclear Magnetic resonance was used to determine the purity and structure of the samples by applying a strong magnetic field to it. There will be the nuclear spin of the sample when it present under a strong magnetic field region. NMR is a non-destructive sample analysis and only required in a small amount of sample.

Around 10 mg of the sample was dissolved in deuterated dimethylsulfoxide in a sample vial. The dissolved sample was then transferred into the NMR tube until 4 cm height. The deuterated solvent is to avoid the interrupt of the proton in the solvent.

3.4.4 Melting Point Apparatus

Melting point apparatus is used to measure the sample melting point. A small amount of the sample was put in a capillary tube. Then the capillary tube was inserted into the melting point apparatus. The temperature has to set at a higher value. As the temperature increase, the changes of the sample can be observed through a magnifying lens on the apparatus. The melting point is measured when the sample starts melting and completely melted.

3.5 Calculations

1. $Mass(g) = number\ of\ mole(mol) \times Molar\ Mass(gmol^{-1})$

Use to calculate the mass of the starting materials.

2. $Volume = mass(g) \times density(gcm^{-1})$

Use to convert the mass of starting materials to volume required in synthesis.

3. $Percentage\ yield = \frac{Actual\ mass\ obtained(g)}{Theoretical\ mass\ (g)} \times 100\%$

Use to calculate the product percentage yield.

CONCLUSION

From this project the carboxylic acid hydrazide and four type of 1,3,4-oxadiaozles were being synthesized. The carboxylic acid hydrazide was synthesized by reacting the indole ester with the hydrazine hydrate. And the carboxylic acid hydrazide is the starting material of the 1,3,4-oxadiazoles. The percentage yield of carboxylic acid hydrazide synthesized is 35%. By reacting the carboxylic acid hydrazide with various benzoic acid derivatives in the presence of phosphorous oxychloride can synthesis different 1,3,4-oxadiazoles. The characterization methods being used were ^{1}HNMR, ^{13}C NMR, DEPT, HMQC and HMBC.

FURTHER STUDY

Various journals had stated that the 1,3,4-oxadiazoles have many biological and pharmaceutical properties because of their heterocyclic structure. Therefore, we can study biological activities such as antioxidant activity, antibacterial, antifungal or

anticancer activity. By reacting the carboxylic acid hydrazide with different substituted benzoic acid, this can produce several different 1,3,4-oxadiazoles.

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